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Adding Enriched Eggs in Ready-to-use foods Improve Recovery Rate in Malnourished Rats

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Abstract

Ready-to-use foods (RUFs) using indigenous sources in developing countries is highly required to treat moderate acute malnutrition (MAM). However, incorporating an animal protein may affect their effectiveness. Thus, two local RUFs were produced without (LF-1) and with eggs (LF-2). The objective of this study was to assess and compare to *Plumpy'Sup* (PS), the impact of adding enriched eggs in cashew/soy/rice based RUF on the proximate composition, growth and blood biochemical parameters in malnourished *Wistar* rats by Anagobaka diet. Proximate composition revealed that, with the exception of fiber and ash contents, the two RUFs recorded protein, lipid, carbohydrate and energy values globally comparable to PS. They also met WFP's recommendations for foods to treat MAM. Results of growth parameters show that Anagobaka diet leads to the installation of a moderate emaciation, confirmed by an average weight loss of -17 %. Moreover, recovery diets showed higher weight gain and good palatability (DMI, TPI, FER and PER) in rats fed with PS followed by those fed with LF-2 and LF-1. For the serum biochemical parameters, the rats fed with LF-2 had on the whole a better functioning of blood metabolites (glucose, total proteins, albumin, urea, creatinine, ASAT, ALAT) as well as a better accumulation of blood lipids (total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides) than those of rats fed with PS and LF-1. In conclusion, local RUFs which include enriched eggs present the best nutritional profile to treat MAM in Côte d'Ivoire but to sustain recovery a mineral supplementation will be needed.

Keywords: Cashew/soya/rice based ready-to-use foods, moderate acute malnutrition, enriched egg, *Wistar* rats, Anagobaka, Côte d'Ivoire

1. Introduction

Developing countries are still affected by infant malnutrition despite the effort made by government and NGO's. In these countries, feeding diversification period is often marked by inappropriate complementary feeding practices (Black, Makrides, & Ong, 2017). Indeed, African mothers especially mothers in Côte d'Ivoire nourished their child with porridge rich in carbohydrate but, poor in protein, energy (Bamba, Gbogouri, Agbo, Digbeu, & Brou, 2018) and unbalanced in micronutrients. This is generally the case of a low cost composite flour called "Anagobaka" (E. Kouakou et al., 2016a) used by mothers registered at National Institute of Public Health in Côte d'Ivoire whose child are moderate or severely malnourished.

Thus, these feeding practices cause child emaciation (acute malnutrition) which moderate form in Côte d'Ivoire is more prevalent than severely one (Institut National de la Statistique [INS], 2017). Moreover, in order to limit the progression toward severely form, the management of moderate acute malnutrition (MAM) should be a public health priority (Yepié et al., 2019).

To combat this form of malnutrition, specialized food products called ready-to-use foods (RUFs) are designed. As an example, *Plumpy'Sup* have been developed and successfully used for the treatment of MAM in children (Lazzerini, Rubert, & Pani, 2013). However, their high cost, as well as the recurrent stock breaks observed in the care structure, jeopardize the future of many children who are tomorrow's succession.

In order to overcome these problems, the use of locally raw materials is one of the best sustainable feeding approaches to fight against public health disorders (Toledo & Burlingame, 2006). This is why international organizations FAO/WHO/WFP recommend the production of RUFs based on available local ingredients.

Nevertheless, the optimal quality, quantity, and source of protein used in these RUF to optimize nutritional outcomes and survival is still debated (Noriega & Lindshield, 2014). Studies suggest that animal protein - as opposed to plant-based protein - increases lean body mass, accelerates linear growth, and improves recovery outcomes in undernourished populations (Grillenberger et al., 2003; Murphy & Allen, 2003; Oakley et al., 2010).

Although dairy protein and its byproducts (whey protein) have been previously used and known to be important for growth (Dewey, 2013; Stobaugh et al., 2016), its cost and availability in our local context can be an obstacle regarding its necessity specifically in the treatment of MAM. So, finding other source of animal protein is still the way to explore.

Despite its popularity and low cost, the use of egg in supplementary foods for malnourished children is limited because of its high content in cholesterol and saturated fatty acids. But, reducing these contents is the way to improve the quality of egg and its use in RUF products.

In this preclinical study, we assess the nutritional performance of two RUFs made with local ingredients compared to *Plumpy'Sup* in the rehabilitation of moderately undernourished *Wistar* rats with "Anagobaka" diet.

2. Material and Methods

2.1 Material

2.1.1 Housing of Rats

Twenty four (24) weanling male *Wistar* rats aged between 5 and 6 weeks, with an average weight 60.76 ± 10.45 g were used for the study and housed at Animal Room, Ecole Normale Superieur (ENS), Cocody, Abidjan (Côte d'Ivoire). Relative humidity (between 70 and 80 %), temperature (25 ± 3°C) and light conditions (12 hours' light-dark cycles) were maintained throughout the study period.

2.1.2 Diets

Five diets have been submitted to the growing rats. The first one is the basal diet having fish as source of protein, prepared according to E. Kouakou et al., (2016a) containing 1 % mineral-vitamin mixture. The second one is a composite flour called Anagobaka (milk custard). Two ready-to-use foods (RUFs) have been produced using local ingredients. The fifth diet is the control diet (*Plumpy'Sup*) given by Nutriset® (Malaunay, France).

For the production of RUF, Cashew nut paste (*Anacardium occidental* L.) and polished rice (*Oriza sativa*, var. Bouake) are respectively bought at SARAYA® factory from Bouaké and CODERIZ® factory from Adzopé (Côte d'Ivoire). Poultry eggs enriched in polyunsaturated fatty acids using *Euphorbia heterophylla* seeds (N'G. Kouakou et al., 2015) are produced at the farm of breeding center of Institut National de Formation Professionnelle Agricole (INFPA) de Bingerville (Côte d'Ivoire). Soya seeds (*Glycine sp*), powder sugar and refined palm oil are bought at the local market in Abidjan, Côte d'Ivoire.

2.1.3 Formulation of RUFs

The theoretical formulation of RUF components was made based on linear programming (Yepié et al., 2019) to identify the combinations of ingredients that cover the most nutritional requirements for children between 6 and 59 months of age suffering from moderate acute malnutrition (World Food Program [WFP], 2016). Thus, two RUFs are formulated and called local formula 1 and 2 (LF-1 and LF-2). LF-1 is formulated using only vegetable ingredients while LF-2 includes in its formulation an animal protein ingredient (egg) (Table 1).

Table 1. RUF formulations

Ingredients (%)	LF-1	LF-2
Rice flour	22	23
Soya flour	30	26
Cashew paste	17	17
Egg powder		03
Refined palm oil	21	21
Ice sugar	10	10
Total	100	100

Note. LF-1=Local Formula based on cashew/rice/soya; LF-2=Local Formula based on cashew/rice/soya/egg

2.2 Methods

2.2.1 Treatment of Ingredients

Rice grains and soya seeds have been cleaned (sorting, winnowing and washing 3 times with water). Cleaned rice grains have been successively precooked in the microwave oven (AKAI®, MW04A-23UG20W) for 3 min, roasted in a pan between 120 and 130 ℃ for 30 to 40 min (Ahmed et al., 2014), pulverized in a mill (PHILIPS®, HR2056) and then sieved using a sieve of 150 µm mesh to obtain rice flour. For cleaned soya seeds, it has been soaked in water containing 1 % of sodium bicarbonate (Niraula, 2017). The soaking was carried out in a seed/water ratio 3:10 (w/v) for 8 hours (El-Adawy, Rahma, El-Bedawy, & Sobihah, 2000). The soaked seeds were then drained, skinned and then precooked in the microwave oven for 3 min. The precooked seeds were finally and respectively roasted in a pan between 120 and 130 ℃ for 50 to 70 min, cooling, grinding using a mill and sieving to 300 µm mesh to obtain soya flour.

Icing sugar was obtained by crushing white powdered sugar using a mill and then sieved using a sieve of 150 µm mesh. Egg powder was obtained from chicken eggs. These were first broken to remove the shells. The liquid obtained was homogenized in a multifunction mixer (SOKANY®, KF-103S). It was immediately dried by spreading the liquid on aluminum trays using an oven at 45 ℃ for 24 to 48 hours. The dried eggs were ground using a mill and then sieved with a sieve of 300 µm mesh. Cashew paste and refined palm oil were used without any treatment or transformation.

2.2.2 Preparation of RUF

The preparation of RUF is inspired by the methods described by Ahmed et al., (2014) and Ordiz et al., (2015). The different ingredients (rice and soya flours, cashew paste, egg powder, ice sugar and refined palm oil) have been weighed separately and mixed to obtain a RUF. It was carried out according to the production diagram presented in Figure 1.

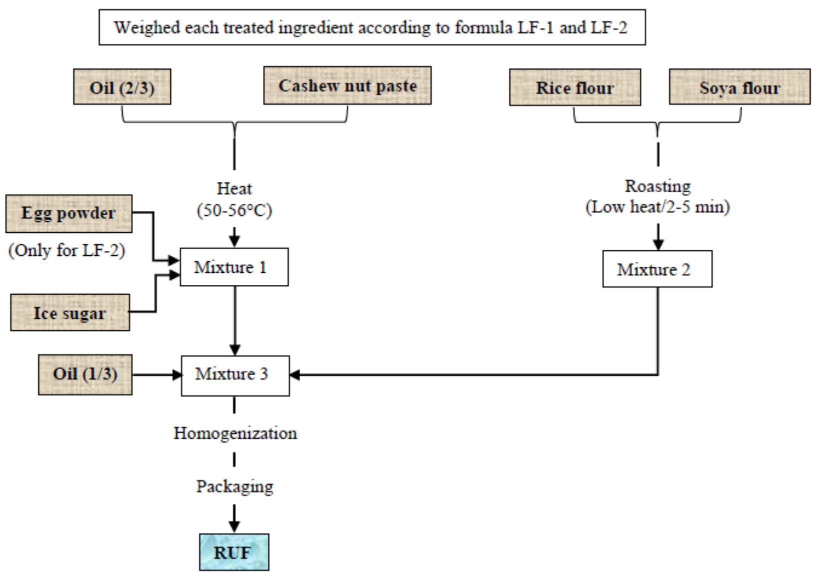

Figure 1. Flow diagram of ready to use foods (RUF) production

2.2.3 Proximate Composition of RUF

The *Plumpy'Sup* and the two (2) newly produced RUF were analyzed for total dry matter, moisture, crude fat, protein, fiber and ash content according to their standard methods described by AOAC, (2000) in triple test. Water activity and pH were respectively measured with Moisture Balance (BM-50-1) and pH-meter (Benchtop/mV meter 210). The carbohydrate content was estimated by differential calculation (FAO/INFOODS, 2015a) while calculation of energy value was carried out according to the relationship given by the conversion coefficient of the metabolized energy also called general factors of Atwater (FAO/INFOODS, 2015b).

2.2.4 Experimental Protocol

It has been done in four phases (Figure 2). In the first one called acclimation phase, rats were fed with basal diet during five days according to 43.254 method (AOAC, 1984). After this period, rats were randomly divided into 4 groups (A, B, C and D) having 06 rats in each group. Group A (06 rats) were continuously fed on basal diet until the end of the experiment. The remaining groups (18 rats) followed the second step called induction of malnutrition phase. These rats were fed during five days with "Anagobaka" a hypercarbohydrate diet to induce malnutrition. Moderate acute malnutrition (MAM) is confirmed when the weight deficit of the rats is between 10 and 39 % according to degree of malnutrition described by Gómez et al. (1946). The third phase is the rehabilitation of 18 moderate malnourished rats. These latters have been rehabilitated in 21 days (E. Kouakou et al., 2016c) with the three following diets: groups B (*Plumpy'Sup*), C (LF-1) and D (LF-2). The fourth phase is called outcome phase. In this phase, nutritional growth and biochemical blood parameters were evaluated.

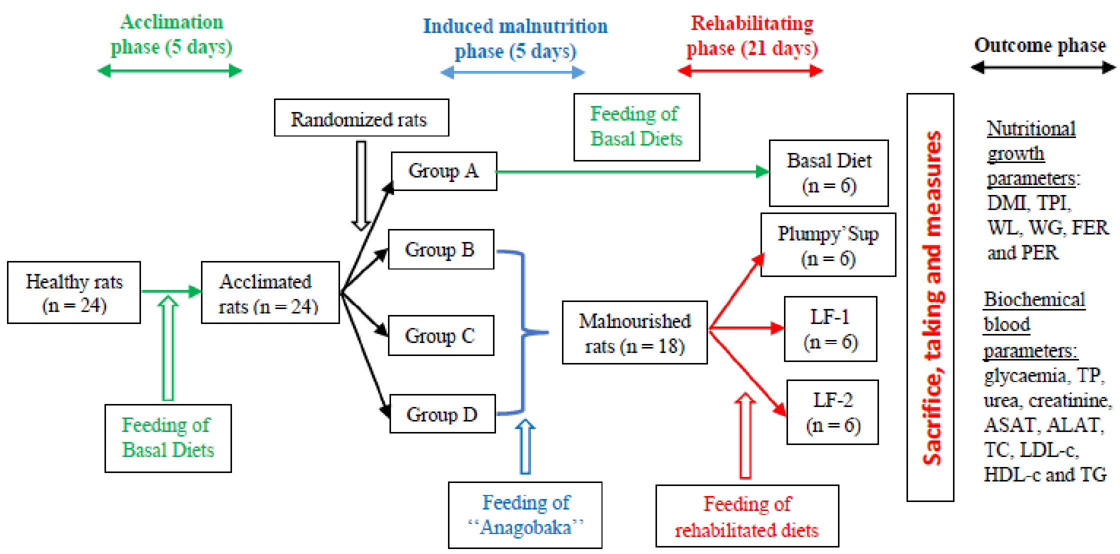

Figure 2. Experimental protocol of preclinical study

2.2.5 Sample Collection and Analysis

The sacrifice took place as stated by the recommendations of the European Commission [EC], (1997) on the euthanasia of laboratory animals. It was carried out according to a terminal procedure by gentle decapitation after ether anesthesia (Descat, 2002). Thus, the six young rats of each group were sacrificed 4 hours after the start of the last meal according to the modified method of Deglaire, Moughan, Bos, & Tome, (2006) under anesthesia in the morning between 11 a.m. and 1 p.m. after having undergone a fast of 12 hours the day before. The blood was then collected for each rat in dry (red) tubes. These tubes were used for the assay of serum biochemical parameters. Once in the laboratory, the tubes were centrifuged at 3000 rpm for 5 min to obtain the serum which was stored at -20 ℃ in eppendorf tubes. The biochemical analyzes were carried out on these serum using a Coulter ACT diff 2 type analyzer, France.

2.2.6 Effectiveness of RUF on Wistar Rats

The novel cashew based RUF was evaluated in comparison to *Plumpy'Sup* in various groups of *Wistar* rats. Nutritional value were evaluated on the basis of weight loss or gain (WL or WG), dry matter ingested (DMI), total protein ingested (TPI), feed efficiency ratio (FER) and protein efficiency ratio (PER) (Table 2) whereas biochemical blood parameters were consisted of determining glycaemia, total proteins, urea, creatinine, ASAT, ALAT, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides contents in serum.

Table 2. Expression of nutritional parameters

Parameter	Mathematical Expressions
DMI (g/d)	Total amount of dry matter ingested during the experimental period
TPI (g/d)	TPI = DMI x % Protein of diet
WL or WG (g/d)	Final weight – Initial weight / number of days
FER	FER = WG / DMI
PER	PER = WG / TPI

WL: weight loss; WG: weight gain; DMI: dry matter ingested; TPI: total protein ingested; FER: feed efficiency ratio and PER: protein efficiency ratio.

2.2.7 Statistical Analysis

Data was analyzed using R software version 3.5.2 and GraphPad Prism version 7.00. Difference between means of proximate composition, nutritional and biochemical parameters were tested for significance using Student Newman-Keuls (SNK) test at 95 % confidence level ($p<0.05$).

3. Results and Discussion

3.1 Results

3.1.1 Proximate Composition

Table 3 presents the proximate profile of LF-1 and LF-2 compared to that of *Plumpy'Sup* (PS). Analysis revealed comparable total energy profile of the three samples; 539.11, 539.34 and 538.06 kcal from PS, LF-1 and LF-2 respectively. For moisture content, a notable difference was noted between PS (2.05) and both local formula LF-1 (2.41) and LF-2 (2.42). Higher pH level was found in PS (6.25) compared to LF-2 (6.19) and LF-1 (6.17). On the same note, dry matter analysis revealed a higher content for PS (97.95 %) compared to LF-1 (97.60 %) and LF-2 (97.58 %). In terms of ash content, levels varied significantly from 4.7 g/100 g in PS to 1.81 g/100 g in LF-1 and 1.80 g/100 g in LF-2. A superior carbohydrate profile was found for PS (42.24 %) compared to LF-2 (41.19 %) and LF-1 (39.87 %). However, LF-2 had an expressively high water activity (0.49) and protein (15.34 g/100 g) contents than LF-1 (0.41; 15.22 g/100 g respectively) and PS (0.26; 13.86 g/100 g respectively). Higher fat levels were successively found in LF-1 (35.44 g/100 g) followed by PS (34.97 g/100 g) and LF-2 (34.66 g/100 g).

Table 3. Proximate composition of *Plumpy'Sup* and the two local formulae

Parameters	WFP Recommendations		*Plumpy'Sup*	LF-1	LF-2
	Min	Max			
pH	_	_	6.25 ± 0.01^{a}	6.17 ± 0.02^{b}	6.19 ± 0.01^{b}
A_w	_	0.6	$0.26 \pm 0{,}02^{c}$	0.41 ± 0.01^{b}	0.49 ± 0.03^{a}
Moisture (%)	_	2.5* or 5**	2.05 ± 0.07^{b}	2.40 ± 0.18^{a}	2.42 ± 0.04^{a}
DM (%)	_	_	97.95 ± 0.07^{a}	97.60 ± 0.18^{b}	97.58 ± 0.04^{b}
Protein (g/100 g)	11	16	13.86 ± 0.07^{c}	15.22 ± 0.07^{b}	15.34 ± 0.02^{a}
Fat (g/100 g)	26	36	34.97 ± 0.25^{b}	35.44 ± 0.02^{a}	34.66 ± 0.01^{c}
Ash (g/100 g)	_	_	4.70 ± 0.01^{a}	1.81 ± 0.05^{b}	1.80 ± 0.03^{b}
Crude fiber (g/100 g)	_	_	2.18 ± 0.04^{c}	5.25 ± 0.05^{a}	4.59 ± 0.03^{b}
AC (%)	_	_	42.24 ± 0.28^{a}	39.87 ± 0.19^{c}	41.19 ± 0.06^{b}
TE (kcal/100 g)	510	560	539.11 ± 1.11^{a}	539.34 ± 0.98^{a}	538.06 ± 0.12^{a}

Source: * OMS, 2007; ** Santini, Novellino, Armini, & Ritieni, 2013; A_w: Water Activity; DM: Dry Matter; AC: Available Carbohydrate; TE: Total Energy. Results are expressed as the mean value ± standard error of three replicates. For assessing statistical significance, a one-way analysis of variance (ANOVA) followed by the Newman-Keuls test at the threshold of 5% was used. On the same column, the means followed by different superscript letters are significantly different ($p < 0.05$).

3.1.2 Nutritional Growth Parameters

The evolution of body weights of the rats is illustrated in Figure 3. This nutritional assessment shows generally two phases. In the first phase of induction of malnutrition, three groups (B, C and D) of rats fed with the hypercarbohydrate diet (Anagobaka) showed a loss of weight while those (Group A) fed with basal diet (BD) showed a gain of weight. Thus, consumption of Anagobaka results in moderate wasting after five days of experimentation with an average weight loss of -2.07 g/d. Statistical analysis revealed that dry matter ingested (DMI) of rats consuming Anagobaka varied from 4.19 ± 0.98 (Group C) to 5.26 ± 0.79 g (Group B). In the second phase of rehabilitation, the groups of moderate malnourished rats were fed with PS, LF-1 and LF-2. All these diets led to a significant ($p < 0.05$) increase in the weight of rats fed with PS showing the best weight gain followed by rats fed with LF-2 and LF-1. However, no significant difference ($p > 0.05$) was observed in weight gain of rats fed with PS and LF-2.

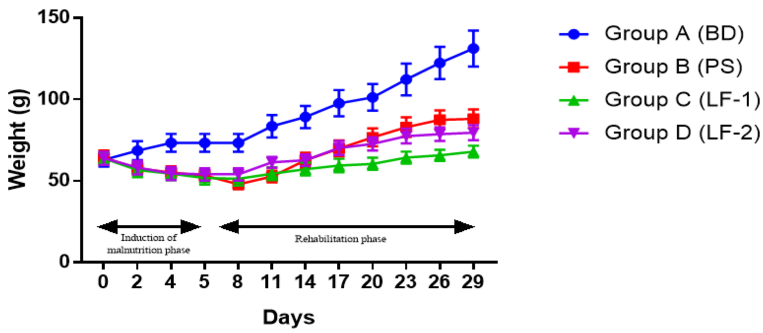

Figure 3. Weight growth of the four groups of rats

Results are expressed as the mean value with standard error mean of 6 rats per group. For assessing statistical significance, a one-way analysis of variance (ANOVA) followed by the Newman-Keuls test at the threshold of 5% was used. BD: Basal Diet, LF-1: Local Formula based on cashew/rice/soya, LF-2: Local Formula based on cashew/rice/soya/egg, PS: *Plumpy'Sup*.

Table 4 presents the nutritional growth parameters of rats after the rehabilitation phase. Analysis showed that there was no significant difference ($p > 0.05$) between initial weights of the different groups of rats in nutritional rehabilitation phase while the final weights of these groups of rats presented significant differences ($p < 0.05$) at the end of the experiment. Significantly higher DMI, TPI and WG levels were found in PS (5.79 g/d, 0.80 g/d and 1.91 g/d) compared to LF-2 (4.45 g/d, 0.68 g/d and 1.22 g/d) and LF-1 (3.75 g/d, 0.57 g/d and 0.79 g/d). On the same note, values of FER and PER are significantly higher in PS (0.33 and 2.39 respectively) than the other diet (BD, LF-2 and LF-1).

Table 4. Nutritional growth parameters of rats after the rehabilitation phase

Parameters	BD (n = 6)	PS (n = 6)	LF-1 (n = 6)	LF-2 (n = 6)
Initial Weight (g)	73,30±12,39^{a}	47,91±6,74^{b}	51,27±9,10^{b}	54,00±8,50^{b}
Final Weight (g)	131,16±24,7^{a}	88,09±12,66^{b}	67,97±8,09^{bc}	79,56±10,25^{b}
DMI (g/j)	11,56 ±1,37^{a}	5,79 ±0,88^{b}	3,75 ±0,37^{c}	4,45 ±0,36^{c}
TPI (g/j)	1,73 ±0,03^{a}	0,80 ±0,12^{b}	0,57 ±0,06^{d}	0,68 ±0,06^{c}
WG (g/j)	2,76 ±0,67^{a}	1,91 ±0,29^{b}	0,79 ±0,11^{c}	1,22 ±0,37^{c}
FER	0,24 ±0,04^{b}	0,33 ±0,04^{a}	0,21 ±0,04^{b}	0,27 ±0,08^{ab}
PER	1,60 ±0,66^{b}	2,39 ±0.26^{a}	1,41 ±0,28^{b}	1,78 ±0,51^{b}

Results are expressed as the mean value with standard error mean of 6 rats per group. For assessing statistical significance, a one-way analysis of variance (ANOVA) followed by the Newman-Keuls test at the threshold of 5% was used. On the same column, the means followed by different superscript letters are significantly different ($p < 0.05$). WG: Weight Gain; DMI: Dry Matter Ingested; TPI: Total Protein Ingested; FER: Feed Efficiency Ratio; PER: Protein Efficiency Ratio.

3.1.3 Biochemical Blood Parameters

The glycaemia, hepatic, renal and lipid outcomes of the young rats subjected to the basal and nutritional rehabilitation diets are recorded in Table 5. With the exception of reference diet (*Plumpy'Sup*), the serum glucose content of the rats subjected to other diets showed no significant difference ($p>0.05$). The highest content is obtained with rats subjected to *Plumpy'Sup* (PS) (2.16 g/L) while other diets have contents varying from 0.13 (BD) to 0.75 g/L (LF-2). For the hepatic outcome, the serum level of albumin and ALAT of rats fed with different diets showed no significant difference ($p>0.05$). However, total protein level of local formula LF-1

(77.10 g/L) and LF-2 (62.59 g/L) was higher than those fed with BD (47.49 g/L) and PS (46.70 g/L). For the serum ASAT level, statistical analysis revealed lower value in LF-1 (212.36 IU/L) than the three other diets (BD, PS and LF-2) which registered no significant difference (*p>0.05*) between them.

Table 5. Biochemical blood parameters of rats fed by rehabilitated diets

Parameters		Dietary regimens			
		BD (n=6)	PS (n=6)	LF-1 (n=6)	LF-2 (n=6)
Glycaemia	Glucose (g/L)	0.13 ± 0.11^{b}	2.16 ± 1.29^{a}	0.18 ± 0.08^{b}	0.75 ± 0.42^{b}
Hepatic outcome	TP (g/L)	47.49 ± 12.60^{b}	46.70 ± 13.68^{b}	77.10 ± 6.95^{a}	62.59 ± 14.68^{ab}
	Albumin (g/L)	28.45 ± 1.63^{a}	29.48 ± 3.25^{a}	30.90 ± 2.12^{a}	29.19 ± 2.11^{a}
	ASAT (UI/L)	353.91 ± 113.37^{a}	417.79 ± 45.7^{a}	212.36 ± 75.27^{b}	371.74 ± 85.12^{a}
	ALAT (UI/L)	177.91 ± 127.27^{a}	205.35 ± 48.35^{a}	143.02 ± 61.44^{a}	186.72 ± 92.18^{a}
Renal outcome	Urea (g/L)	0.51 ± 0.12^{a}	0.46 ± 0.09^{a}	0.55 ± 0.13^{a}	0.57 ± 0.10^{a}
	Creatinine (mg/L)	6.01 ± 1.10^{a}	5.97 ± 0.75^{a}	6.15 ± 1.08^{a}	7.39 ± 1.17^{a}
lipid outcome	TC (g/L)	1.15 ± 0.30^{a}	1.21 ± 0.12^{a}	1.38 ± 0.29^{a}	0.96 ± 0.14^{a}
	TG (g/L)	1.09 ± 0.31^{a}	1.21 ± 0.36^{a}	0.97 ± 0.19^{a}	1.00 ± 0.14^{a}
	HDL-c (g/L)	0.67 ± 0.14^{a}	0.34 ± 0.17^{b}	0.36 ± 0.15^{b}	0.51 ± 0.15^{ab}
	LDL-c (g/L)	0.36 ± 0.26^{b}	0.37 ± 0.19^{b}	1.95 ± 3.38^{a}	0.25 ± 0.06^{b}
	LDL-c/HDL-c	0.54	1.09	5.42	0.49
	TC/HDL-c	1.72	3.56	3.83	1.88

Results are expressed as the mean value with standard error mean of 6 rats per group. For assessing statistical significance, a one-way analysis of variance (ANOVA) followed by the Newman-Keuls test at the threshold of 5% was used. On the same column, the means followed by different superscript letters are significantly different (*p <0.05*). TP: Total Protein; ASAT: Aspartate amino-transferase; ALAT: Alanine amino-transferase; TC: Total Cholesterol; TG: Triglycerides; HDL-c: High Density Lipoprotein cholesterol; LDL-c: Low Density Lipoprotein cholesterol.

As for the renal outcome, statistical analysis of urea and creatinine levels showed no significant difference (*p > 0.05*) between the different regimens. Regarding the lipid outcome, the serum of total cholesterol and triglycerides of young rats which ingested the different diets did not show any significant difference (*p > 0.05*).

For HDL-c, the highest serum concentration is indicated by rats fed with BD (0.67 ± 0.14 g/L) followed by the rats having ingested LF-2 (0.51 ± 0.15 g/L). In addition, the incorporation of egg powders in LF-2 increases values of formula based solely on plant ingredients LF-1 (0.36 ± 0.15 g/L) to that of 29.41 %. However, the statistical analysis did not detect any significant difference (*p > 0.05*) between these values. Conversely, results of LDL-c showed that the lowest serum concentration is recorded in rats having ingested LF-2 diet (0.25 ± 0.06 g/L) followed by BD (0.36 ± 0.26 g/L) and PS (0.37 ± 0.19 g/L). The highest serum concentration is indicated in rats fed with LF-1 (1.95 ± 3.38 g/L). However, with the exception of LF-1 values, no significant difference (*p > 0.05*) was observed between other regimens values. Atherogenic indices (LDL-c/HDL-c and TC/HDL-c) are lower in rats fed with BD (0.54 and 1.72) and LF-2 (0.49 and 1.88) diets compared to those fed with PS (1.09 and 3.56) and LF-1 (5.42 and 3.83) diets.

3.2 Discussion

3.2.1 Proximate Composition

All of the formulae studied met WFP's recommendations for RUFs to treat children suffering from moderate acute malnutrition (MAM). These formulae were characterized by low water activity and low moisture which are comparable to those found by Santini et al., (2013), Ryan et al., (2014) and Weber et al., (2016). These values could be beneficial for a better and longer shelf life.

The protein contents of LF-1 (15.22 g/100 g) and LF-2 (15.34 g/100 g) are higher than that of *Plumpy'Sup* (13.86 g/100 g). These values are lower than those determined by Stobaugh et al., (2016) (17.06 g/100 g) and Weber et al., (2016) (17.60 g/100 g) respectively in RUFs based on soy and whey protein, but fall within the range of 11.42 to 15.6 g/100 g described by Ryan et al., (2014), Stobaugh et al., (2016) and Kohlmann et al., (2019) for RUFs based on whey protein. However, the protein content of our formulae is higher than that determined by Santini et al., (2013) whose values were ranged between 13.4 and 14.1 g/100 g for RUFs based on sesame.

LF-1 formula had higher lipid levels compared to *Plumpy'Sup* (PS) as well as LF-2 formula. These values are within the standard set by WFP (26 to 36 g/100 g). Children suffering from MAM have a high energy need (Amegovu et al., 2013). Therefore, they need a diet rich in fat. These fats are also necessary for the absorption of vitamins A and E (Michaelsen et al., 2009) which are vital for rapid recovery and in reduction of incidence associated with acute malnutrition.

Moreover, the ash contents of the local formulae studied are much lower than the PS. Indeed, PS is supplemented with minerals and vitamins (Nutriset, 2017). It therefore contains a higher mineral content than locally produced formulae. Contrary to this, fiber content of LF-1 is higher than those of LF-2 followed by those of PS. Dietary fiber plays an important biochemical and physiological role in digestion (Amegovu et al., 2013). Unfortunately, due to the limits of obvious evidence on the subject of fiber in children, no limit has been set (Amegovu et al., 2013). However, more deepened preclinical studies should be conducted to establish a standard in this area. As for carbohydrate levels and energy density recorded in all of the formulae, they are adequate to provide enough energy for a child to recover from moderate malnutrition.

Finally, the proximate composition revealed that with the exception of fiber and ash contents, the two RUFs recorded protein, lipid, carbohydrate and energy values globally comparable to PS.

3.2.2 Nutritional Growth Parameters

Growth parameters are indicators of the nutritional efficiency of food (Silva et al., 2005). Results from malnutrition induction phase show that rats fed with the hypercarbohydrate diet (Anagobaka) experience weight loss. This weight loss is due to the low levels of dry matter ingested (DMI) (4.19 ± 0.98 to 5.26 ± 0.79 g/d) and low protein contents of the Anagobaka diet (2.06 ± 0.04 g/100 g DM). This result is in agreement with the conclusions of E. Kouakou et al., (2016a,b) who reported similar protein levels for Anagobaka (2.04 ± 0.03 g/100 g DM) and the basal diet (14.94 ± 0.9 g/100 g DM). However, these authors recorded high rates of DMI for Anagobaka (7.94 ± 0.88 g/d) because their experiment lasted 15 days. This weather must have an impact on the habit of rats fed with this diet. The use of Anagobaka caused moderate wasting in young rats with an average weight loss percentage of -17.23 % (i.e. -2.07 g/d) which are more important than those reported by E. Kouakou et al., (2016b) (-0.97 g/d).

Regarding the results of nutritional rehabilitation phase, all diets lead to a weight gain in rats, but those fed with BD recorded the highest final weight (131.16 ± 24.7 g) and DMI (11.56 ± 1.37 g/d). This is due to the fact that these rats did not undergo the induction phase of malnutrition. However, rats fed with the 2 local formulae recorded DMI levels (3.75 ± 0.37 to 4.45 ± 0. 36 g/d) which were statistically identical to each other. These low levels of DMI could be due to the form of food presented to rats. Indeed, our local formulae are in the form of paste which spreads quickly in the rack and therefore difficult to access. However, the BD diet comes in the form of a compact mash that is easy to use by rats. As for the PS diet, it is also in the form of paste except that in this case the use of emulsifiers in the formulation makes this paste compact; which prevents its spreading as in our own formulae. The results of the DMI recorded in rats subjected to local formulae are also lower than those obtained by Bouafou, Konan, Meite, Kouame, & Kati-Coulibally (2011) (5.45 to 6.47 g/d) and E. Kouakou et al., (2016c) (5.45 to 7.01 g/d).

Moreover, the weight gain observed during rehabilitation phase shows that the rats take up again regular growth; which could indicate a regular development of the cellular metabolism of these animals (Dally, Meite, Kouame, Bouafou, & Kati-Coulibali, 2010). Indeed, Adrian, Potus, & Annie (1998) reported that a food that induces growth of 25 g/month (i.e. 0.89 g/d) is considered a good source of protein. As a result, the LF-2 (1.22 ± 0.37 g/d) would constitute an excellent source of protein in the management of malnourished children. Furthermore, the high growth of rats fed with this diet compared to LF-1 (0.79 ± 0.11 g/d) would confirm the impact of incorporating egg powder on the nutritional value of food. However, weight gain of rats is partly promoted by the ingestion of food. To understand this, feed efficiency ratio (FER) has been calculated, which translates the yield with which food is assimilated by body of rats. Thus, the PS (0.33 ± 0.04) presented the best FER followed by LF-2 (0.27 ± 0.08), BD (0.24 ± 0.04) and LF-1 (0.21 ± 0.04).

Another parameter which is the protein efficiency ratio (PER) for measuring growth was calculated. According to Friedman (1996), proteins with a PER of less than 1.5 are of low protein quality. Those with a PER between 1.5 and 2 are of intermediate quality, and when it is greater than 2, the proteins are of good quality. Based on these criteria, protein in LF-1 (1.41 ± 0.28) is of low quality while those of BD (1.60 ± 0.66) and LF-2 (1.78 ± 0.51) diets are of intermediate quality. On the other hand, protein in PS (2.39 ± 0.26) is of good quality. However, a low PER value does not always indicate a poor ability of the protein to ensure growth. As there is divergence of amino acids requirements between humans and rats particularly for sulfur amino acids, the calculation of PDCAAS or DIAAS (Digestible Indispensable Amino Acid Score) is highly recommended (FAO, 2013;

Callaghan, Oyama, & Manary, 2017).

Consequently, dietary factors, in particular dietary fiber, play an important role in this low PER value observed in LF-1 which fiber content was 5.38 g/100 g DM. Boisen and Eggun (1991) show that dietary fiber influences the digestion of proteins by reducing the activity of enzymes in digestive tract. In addition, due to their high water retention capacity, the fibers exert a "barrier" effect which would slow down the digestion of food proteins (Leterme, Rossi, & Thewis, 1997) and the reabsorption of digestive secretions. All this justifies the higher weight gain of LF-2 (4.70 g/100 g DM), PS (2.23 g/100 g DM) and BD (0.83 g/100 g DM) because they have lower fiber contents than LF-1. This study therefore makes it possible to conclude that a quantity of dietary fiber ≤ 5 g/100 g DM would be indicated when formulating RUFs for the management of moderate acute malnourished children.

3.2.3 Biochemical Blood Parameters

The evaluation of biochemical blood parameters is an important step in human nutrition to remove any ambiguity about the dangers relating to consumption of food (Adeyemi, Odutola, Olugbenga, Funmilayo, & Sunday, 2015). In fact, these parameters are substances synthesized by the body whose excess or deficit in production is indicative of its dysfunction (Kouadio, Kra, & Niamke, 2017). Moreover, postprandial hyperglycemia and hyperlipidemia are nowadays recognized as risk factors for cardio-metabolic diseases (O'Keefe & Bell, 2007) and the reduction of these postprandial excursions is notably proposed to reduce the risk of developing such pathologies.

Thus, the low blood glucose values of rats fed with local diets (LF-1 and LF-2) did not undergo large variations compared to values of rats fed with BD. But, rats fed with PS diet (2.16 ± 1.29 g/L) recorded the highest value. Therefore, the consumption of local diets is without risk on the postprandial glycaemia of young rats; which suggests that their regular consumption reduces the risk of developing metabolic diseases in adulthood. These results could be justified on the one hand, by the presence in the local diets of polyphenols and/or fibers responsible for this antihyperglycemic action (Jenkins, Wolever, & Leeds, 1978; Khan et al., 2011) and on the other hand, by early consumption of the food served. However, the hyperglycemia observed in rats fed with PS diet could be explained by a late consumption of food served, which makes it possible to measure the glycaemia for these rats in the hyperglycemic peak phase.

The fact that there is no significant difference ($p > 0.05$) on the one hand, between the uremia and on the other hand, between the creatininemia of rats fed with BD diet and those of rats subjected to local and reference diets would attest the good renal functioning in these animals (Bankir, 1986). Our values are comparable to those found by Kouakou et al., (2006a) for the control diet (0.52 ± 0.13 g/L) and the “Cerelac Ble” diet (0.71 ± 0.05 g/L). All these observations demonstrate the harmlessness of foods formulated on renal functioning of rats.

The evolution of plasma lipid profile after consumption of diets, shows no significant difference ($p > 0.05$) between the total cholesterol and triglyceride contents in the rats fed with local and reference diets compared to those of rats fed with BD diet. This could be due to intrinsic factors to each formula such as the vegetable origin of ingredients used because they are devoid of cholesterol (Guthrie, 1989), the presence of fibers and tannins known for their impact on the reduction of cholesterol and triglycerides in the blood (Mami, 2015; Dakia, Combo, Yapo, Brou, & Paquot, 2017) and the quantity and quality of lipids as well as their structure and organization in food (Vors, Nazare, Michalski, & Laville, 2014). Regarding HDL-c, their levels in rats fed with diets are statistically variable but comparable to those reported by Goutianos et al., (2015) of 0.55 g/L for normal rats. Also, adding egg powder in LF-2 formula would have increased their good cholesterol levels. As for LDL-c, their levels in rats fed with diets are statistically comparable with the exception of LF-1 which has a high level (1.95 ± 3.38 g/L). Consumption of this formula could lead to a risk of hyperlipidemia or cardiovascular disease (CVD) in children.

However, TC/HDL-c ratio is twice as instructive (Lewington et al., 2007) of the individual risk of CVD than the TC or LDL-c. So, variations of this ratio in populations is mainly due to lifestyle factors (diet, physical activity, obesity, alcohol consumption). TC/HDL-c ratio is probably the most reliable measure of lipids to assess the risk of CVD linked to diet. Thus, the low TC/HDL-c ratios observed with BD (1.72) and LF-2 (1.88) diets indicate that they present less risk than PS (3.56) and FL-2 (3.83) diets. This indicates that the addition of enriched egg in plant based RUF formula has positive impact on the blood lipid accumulation. Moreover, the HDL-c/LDL-c atherogenicity ratios of diets confirm those of TC/HDL-c ratios with always the BD (0.54) and LF-2 (0.49) diets presenting less risk than PS (1.09) and LF-1 (5.42) diets.

4. Conclusion

This study was conducted to assess the nutritional performance of two RUFs made with local ingredients in the rehabilitation of moderately undernourished *Wistar* rats with “Anagobaka” diet. To achieve this, proximate composition of RUFs was conducted followed by its nutritional efficiency in rats.

Results of proximate composition revealed that with the exception of fiber and ash contents, the two RUF formulae (LF-1 and LF-2) had protein, fat, carbohydrate and energy values globally comparable to the reference diet (*Plumpy'Sup*). They also meet the WFP recommendations for MAM diets. Furthermore, the results of the nutritional growth parameters allow us to conclude on the first hand that, the hypercarbohydrate diet (Anagobaka) leads to the installation of moderate wasting, confirmed by an average weight loss of -17.23 %. On the other hand, results of nutritional rehabilitation phase show a higher weight gain and better palatability (DMI, TPI, FER and PER) in rats particularly fed with *Plumpy'Sup* (PS) than those fed with LF-2 and LF-1 formulae. As for the serum biochemical parameters, rats fed with LF-2 show on the whole a better functioning of blood metabolites (glucose, total proteins, albumin, urea, creatinine, ASAT, ALAT) and better accumulation of blood lipids (total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides) than those fed with PS and LF-1 formula.

In view of these results, ready-to-use foods made from local resources which include enriched eggs (LF-2) present the best nutritional profile to treat MAM in Côte d'Ivoire but to sustain recovery a mineral supplementation will be needed.

Competing interests

The authors declare no competing interests in this study.

Author contributions

A.H.Y. conceived the project, wrote the manuscript and performed, analyzed or designed all experiments in this manuscript. I.C. and N.L.A. helped conceive the project, improved the versions of the manuscript. A.M. revised the project and supervised the animal experiments. O.S.A.-T. and A.L.A.A. are mentors of the project and supervised it. All authors have read and approved the final version of the manuscript.

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