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Impacts of Ivorian Food Products Based on Local Ingredients (PASLoc) Used for Nutritional Rehabilitation on the Growth and Biometrics of Organs in Malnourished Rats

Viviane Jocelyne Zannou Tchoko, Brou Andre Konan*, Alassane Meite

Laboratory of Biology and Health, Felix Houphouet-Boigny University, Abidjan, Ivory Coast

Email address:
akonanb@yahoo.fr (Brou Andre Konan)

*Corresponding author

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Abstract: PASLoc are therapeutic foods based on local products used for nutritional rehabilitation in Côte d'Ivoire due to the difficulties of supplying food products approved by UN institutions. Experimental nutritional studies had not been done before their use. Therefore, the present research was conducted to investigate the effects of 3 PASLoc diets on food intake and biometrics of regulatory organs in malnourished rats. Induction of malnutrition in rats by Anagobaka caused weight loss of -24.42% to -28.46%. A weight gain of 31.90 - 51.22% in malnourished rats having received the PASLoc diets was recorded against 79.49% for the Plumpynut (reference diet). This weight gain is underpinned by positive values of the consumption indices. The relative weight values showed that, except for the kidneys, there was no difference in the liver, heart, spleen and abdominal fat of the rats fed the different rehabilitation diets. Like Plumpynut, PASLocs have allowed the rehabilitation of malnourished rats. These results testify to the potential of PASLoc and their use as a substitute for Plumpynut for nutritional rehabilitation.

Keywords: Nutritional Rehabilitation, Malnourished Rat, Specialized Food Products, Local Products, Plumpynut

1. Introduction

Although things have improved, the nutritional situation in Côte d'Ivoire remains a concern in the North, North East, North West, West, South West and Center North regions according to the 2015 National Nutrition Program report. And this situation is more worrying during seasonal fluctuations and especially in periods of food deficit [1]. The prevalence rate of malnutrition due to deficiency is critical. Global chronic malnutrition affects 38% of children. As for acute malnutrition, the overall prevalence is 6%. This situation requires urgent care that includes a phase of nutritional recovery. During this phase of nutritional rehabilitation, the child is fed intensively to compensate for most of the lost weight so that he can recover quickly and escape death, the ultimate outcome of acute malnutrition if nothing is done to save him.

The use of Ready-to-Use Therapeutic Nutritious Foods (RUTF) is therefore recommended by WHO, UNICEF and WFP against malnutrition. These are Plumpynut, Plumpysup, CSB, F-100 and F-75. These are specialized food products (SFP), made available to nutritional services, health personnel and people suffering from malnutrition. These products have proven their effectiveness in the nutritional rehabilitation of malnourished people. However, recurrent stock-outs, difficulties in accessing supplier services and the high unfulfilled demand from healthcare providers compromise the nutritional treatment of patients and lead to the abandonment of users [1]. This is why the Ministry of Health and Public Hygiene (MSHP) of Ivory Coast through the National Nutrition Program (PNN) has opted for food formulas based on local ingredients available and accessible. The main objective of this initiative is to offer specialized substitute food products based on local products (PASLoc) as an alternative to RUTF in order to contribute to the nutritional rehabilitation of patients. These PASLocs are mixtures of cereal flours (corn, millet, sorghum, rice),

soybeans, peanuts, fish powder, milk powder, sugar and refined palm oil. Formulated according to UNICEF and WHO standards (13 – 14% protein and 400 - 480 Kcal of energy value), these foods based on local products could be effective in the fight against malnutrition [2, 3]. However, the question that arises is whether these foods, although already used for the rehabilitation of malnourished people, could really and effectively replace specialized foods approved by UN agencies.

It is in this context of evaluating the beneficial potential of PASLoc through a scientific approach that the present study is located. It was carried out in order to examine the effects of PASLoc consumption on growth parameters and organs regulating nutrition in undernourished rats.

2. Material and Methods

2.1. Animals and Ethics

24 young Wistar rats weighing 54,6 ± 0,5 g and aged 8-week-old were used. They were obtained from the *Vivarium* **(**animal house), Ecole Nationale Supérieure (ENS), Abidjan, Côte d'Ivoire. Each was housed in metabolic cages and maintained under standard laboratory conditions (temperature 25 ± 2°C) with dark and light cycle (12/12 h). They were allowed free access to standard dry pellet diet and water *ad libitum*. Rats were treated according to good laboratory practices [4]. The experimental protocols were conducted in accordance with the protocols for the protection of experimental animals of the European Council on Legislation 2012/707 [5].

2.2. Food Material

Four local products (corn, millet, soy and groundnuts), milk, sugar, refined palm oil, Anagobaka flour and Plumpynut have been constituted the food material. Except for the Plumpynut supplied by Nutriset, all the other ingredients were sourced from local markets. Plumpynut is a widely used reference SFP for nutritional rehabilitation. Local products (corn, millet, soybeans and peanuts), milk, sugar and refined palm oil were used to formulate PASLoc. Anagobaka is a low protein infant flour and it has been used to induce malnutrition in rats [6, 7].

2.3. Formulation of PASLoc Diets

The PASLoc were obtained in two steps in strict compliance with the PNN recommendations. The first step consisted in the preparation of flours (corn, millet, soy) and groundnut paste, while the second was the formulation of the PASLoc diets. Maize, millet and soy grains were separately cleaned, freed of all impurities and washed with water. They were then rehydrated and allowed to germinate. After dehulling, the grains were crushed, dried, sieved and roasted. Cooled in the open air, the flours obtained were stored away from humidity. Ripe and dry groundnut seeds were cleaned, shelled and dehulled. Grilled over low heat and peeled, the seeds were finely ground. The paste obtained was packaged in hermetic glass containers in the open air.

Three PASLocs were formulated from flour, groundnut paste and other ingredients in the proportions shown in table 1. These are the Corn-Milk diet (MaLait), the Millet-Soy diet (MiSoj) and the Corn-Soy-Groundnut diet (MaSojAr).

2.4. Chemical Compositions and Energy Values of Diets

PASLoc (MaLait, MiSoj, MaSojAr), Plumpynut and Anagobaka were the five diets studied. These diets contained protein (2 - 18%), lipids (6.04 - 33%) and carbohydrates (27.50 - 82.10%). The energy values of the diets varied between 390 and 574.40 Kcal (Table 2).

2.5. Preparation of Diets

Except Plumpynut which is a RUTF, PASLoc and Anagobaka were subjected to cooking. At the rate of 100 g of flour for 50 ml of water, the Anagobaka flour and each PASLoc (mixture of ingredients) were separately mixed in water and brought to a low heat with constant stirring. After 5 min of cooking, the purees obtained were left in the open air to cool before being served to the animals. Purees were prepared daily.

Table 1. *Proportions of ingredients used for the formulation of PASLoc.*

PASLoc diets	Proportions of ingredients (%)						
	Corn flour	Millet flour	Soy flour	Groundnut paste	Milk	Sugar	Refined palm oil
MaLait	35	-	-	-	35	24	6
MiSoj	-	52	26	-	-	9	13
MaSojAr	20	-	20	40	-	10	10

PASLoc: Specialized food products based on local ingredients, MaLait: Corn-Milk, MiSoj: Millet-Soy, MaSojAr: Corn-Soy-Groundnut.

Table 2. *Chemical compositions and energy values of test diets.*

Parameters	Anagobaka	Plumpynut	PASLoc		
			MaLait	MiSoj	MaSojAr
Proteins (%)	2.04	15.29	13	14	18
Lipids (%)	6.04	37.30	17	22	33
Carbohydrates (%)	82.10	44.47	57	39	27.50
Energy values (Kcal)	390.92	574.40	433	410	479

PASLoc: Specialized food products based on local ingredients, MaLait: Corn-Milk, MiSoj: Millet-Soy, MaSojAr: Corn-Soy-Groundnut. The composition and energy value of the Anagobaka diet were determined by Egnon [7].

2.6. Experimental Design and Measurement of Biological Parameters

The Following the method of Egnon *et al.* [8], animal experimentation lasted 30 days. It was conducted in two stages (Malnutrition phase and Nutritional rehabilitation phase). During the 14 days of the malnutrition phase, the 24 rats, divided into 4 groups of 6 animals, were all fed daily with the Anagobaka diet. Rats were weighed at the start and end of the phase. Intermediate weighings at regular intervals of 2 days were also made. The quantities of food served and refusals (food remaining after consumption) were measured. The data collected made it possible to assess different biological parameters of consumption. These are body weight, weight gain (WG), total dry matter ingested (DMI), total protein ingested (TIP), feed efficiency ratio (FER) and protein efficiency ratio (PER). At the end of this phase, one rat chosen at random from each group was sacrificed. Liver, heart, spleen, kidney and abdominal fat were sampled for biometric observations (morphology and calculation of relative weights).

The nutritional rehabilitation phase lasted 16 days. The 5 malnourished rats of group 4 received Plumpynut daily when those of groups 1, 2 and 3 were fed respectively with Malait, MiSoj and MaSojAr diets. Following the logic of the previous phase, various weighings were carried out with a view to measuring the same biological consumption parameters. One day after the 16th day of nutritional rehabilitation (end of phase), all animals were sacrificed. Abdominal fat and organs were sampled for biometric parameter measurements.

2.7. Data Analysis

The values were expressed as mean with standard error of the mean (m ± sem). The data were evaluated by analysis of variance followed by Tukey-Kramer with GraphPad Instat software (Microsoft, San Diego, California, USA) method. The graphical representations of data were performed by the GraphPad Prism 5 software (Microsoft, San Diego, California, USA). The difference between the averages is considered statistically significant when $p < 0.05$.

3. Results

3.1. Impacts of Test Diets on Biological Consumption Parameters in Rats

3.1.1. Evolution of the Body Weight

The Anagobaka diet caused weight loss during the malnutrition phase in animals (Figure 1a). The mean weight of rats given the Anagobaka diet had decreased from 55.10 ± 3.93 to 39.42 ± 3.68 g (Group 1), 55 ± 4.30 to 41.57 ± 4.12 g (Group 2), 54.90 ± 3.99 at 39 ± 2.50 g (Group 3) and 53.50 ± 0.75 at 40 ± 1.50 g (Group 4) corresponding to respective rates of -28.46%, -24.42%, -28.96% and -25.23%. Conversely, during the rehabilitation phase, effective weight regain was observed in all rats having consumed the Plumpynut and the PASLoc diets (Figure 1b). Indeed, the PASLoc MaLait, MiSoj and MaSojAr diets served respectively to the malnourished rats of groups 1, 2 and 3 increased the average weight of the animals in the same order from 39.42 ± 3.68 to 59.61 ± 5,10 g, 41.57 ± 4.12 to 60.94 ± 4.29 g and 39 ± 2.50 to 51.44 ± 3.99 g. The relative values of these weight regains were estimated at 51.22% (MaLait), 46.60% (MiSoj) and 31.90% (MaSojAr). A greater weight regain (79.49%) was recorded in the malnourished rats of group 4 which had received Plumpynut. The mean weight of the rats in this group increased from 40 ± 1.50 to 71 ± 1.17 g.

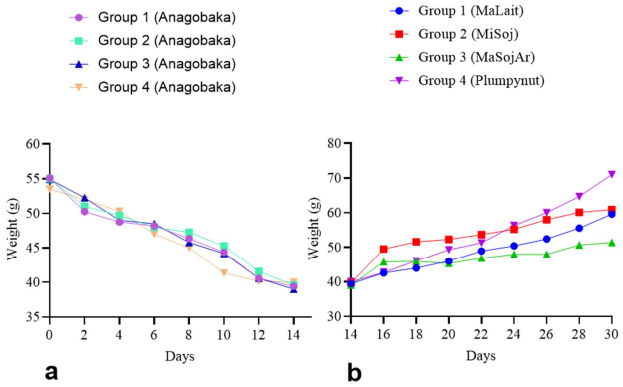

Figure 1. *Effects of formulated diets on the body weight of rats. a) Malnutrition phase, b) Nutritional rehabilitation phase. Anagobaka caused weight loss in rats while the MaLait, MiSoj, MaSojAr and Plumpynut diets allowed weight regain in animals. m ± sem, n = 5-6, MaLait: Corn-Milk, MiSoj: Millet-Soy, MaSojAr: Corn-Soy-Groundnut.*

3.1.2. Dry Matter Ingested

The values of total dry matter ingested obtained were 3.74 g (MaLait), 3.6 g (MiSoj) and 2.99 g (MaSojAr). The Plumpynut reference diet was consumed at 3.24 g against 3.68 g for the Anagobaka diet (Table 3).

Table 3. *Influence of test diets on total dry matter and proteins ingested by rats.*

Diets		Parameters	
		TDM/D (g)	TPI/D (g)
Anagobaka		3.68	0.08
Plumpynut		3.24	0.50
PASLoc	MaLait	3.74	0.48
	MiSoj	3.60	0.49
	MaSojAr	2.99	0.52

TDM/D: Total dry matter ingested per day, TPI/D: Total proteins ingested per day, PASLoc: Specialized food products based on local ingredients, MaLait: Corn-Milk, MiSoj: Millet-Soy, MaSojAr: Corn-Soy-Groundnut.

3.1.3. Total Proteins Ingested

Total proteins ingested (TPI) were 0.4 g for rats fed MaLait. TPI values of 0.49 g and 0.52 g were determined in the rats which had consumed MiSoj and MaSojAr respectively. The protein amount of Plumpynut ingested was 0.50g and that of Anagobaka was 0.08 g (Table 3).

3.1.4. Weight Gain

The Anagobaka diet, low in protein, caused in the rats an

average weight loss of -1.12 g/d. Conversely, the nutritional rehabilitation diets studied have favored positive weight gains. The weight gain values measured were 1.26 ± 0.56 g/d, 1.21 ± 0.3 g/d and 0.77 ± 0.43 g/d for the rats fed respectively with PASLoc MaLait, MiSoj and MaSojAr. The Plumpynut reference diet allowed the highest weight gain (1.93 ± 0.6 g/d). The difference in value obtained with Plumpynut and the PASLoc Malait and MiSoj was not significant at $p > 0.05$ (Figure 2).

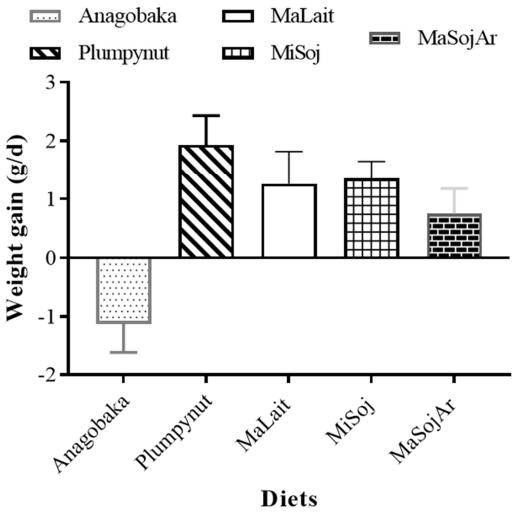

Figure 2. *Effects of test diets on weight gain in rats. m ± sem; n = 5; ****p < 0.00001, **p < 0.001: significant différence compared to Plumpynut; MaLait: Corn-Milk, MiSoj: Millet-Soy, MaSojAr: Corn-Soy-Groundnut.*

3.1.5. Feed Efficiency Ratio

Dietary assimilation of diets indicated 0.36 ± 0.08 for rats fed MiSoj, 0.34 ± 0.15 for rats fed MaLait, 0.26 ± 0.15 for rats fed MaSojAr, and 0.6 ± 0.10 for rats that received the Plumpynut reference diet (Figure 3). Statistical analysis showed a significant difference at $p < 0.05$ between the reference diet and the PASLoc diets.

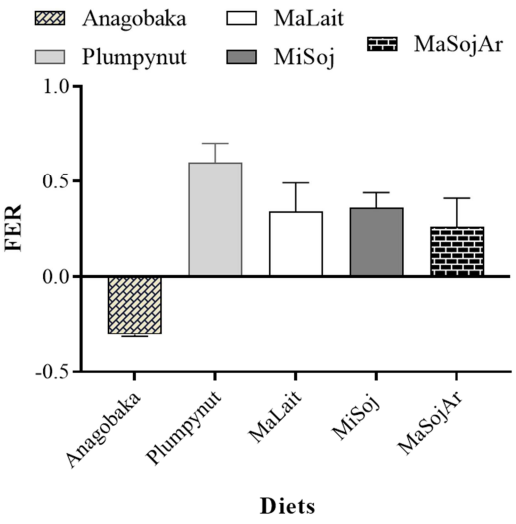

Figure 3. *Effects of test diets on the food efficiency ratio of rats. m ± sem; n = 5; **** p < 0.00001, ** p < 0.001, *p < 0.05: significant différence compared to Plumpynut; FER: Food efficiency ratio, MaLait: Corn-Milk, MiSoj: Millet-Soy, MaSojAr: Corn-Soy-Groundnut.*

3.1.6. Protein Efficiency Ratio

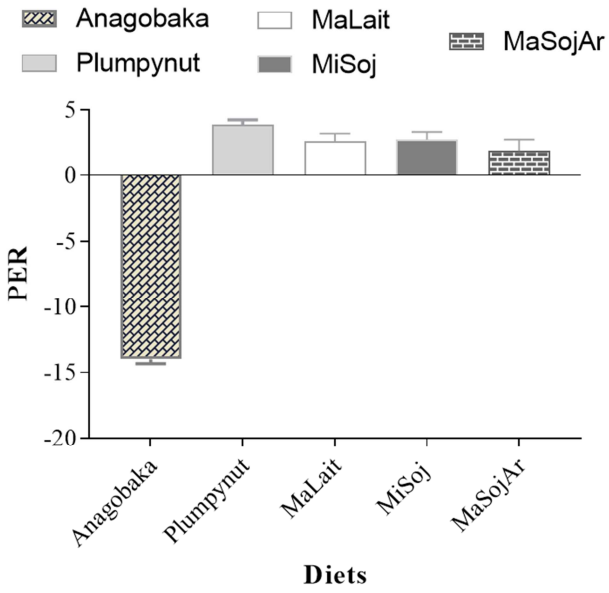

Figure 4. *Effects of test diets on the protein efficiency ratio of rats. m ± sem: n = 5; ****p < 0.00001, **p < 0.001, *p < 0.05: significant différence compared to Plumpynut; PER: Protein efficiency ratio, MaLait: Corn-Milk, MiSoj: Millet-Soy, MaSojAr: Corn-Soy-Groundnut.*

Figure 4 shows the protein efficiency ratio (PER) calculated in rats that consumed the different diets. A PER of 2.62 ± 0.6 was recorded in rats fed with MaLait. The measured value was 2.75 ± 0.60 in the animals fed with MiSoj and 1.44 ± 0.43 in those which had consumed MaSojAr. The highest PER value (3.86 ± 0.4) was obtained in animals fed the reference diet Plumpynut with a significant difference at $p < 0.05$ in comparison to those of PASLoc diets.

3.2. Effects of Test Diets Consumption on the Biometric Parameters of Nutrition Regulatory Organs

3.2.1. Morphology of Organs

Macroscopic examination did not reveal any modification of the removed organs (heart, spleen, liver and kidney) in the rats. Organ shape and color were unaffected by the different test diets given to the animals. No anomaly (hypertrophy, atrophy, presence of nodules) was observed.

3.2.2. Relative Organ Weights

Heart: The relative weights of rat hearts were 0.32 ± 0.05 (Plumpynut), 0.32 ± 0.04 (MaLait), 0.28 ± 0.02 (MiSoj) and 0.29 ± 0.05 (MaSojAr) against 0.24 ± 0.01 for the Anagobaka diet. Statistical analysis showed no significant difference ($p > 0.05$) between Plumpynut and PASLoc diets (Table 4).

Liver: The relative weight calculated was 2.02 ± 0.145 for the rats fed with Anagobaka, 3.26 ± 0.20 for the rats which had consumed MaLait, 2.81 ± 0.22 for the rats fed with MiSoj and 2.44 ± 0.33 for MaSojAr-fed rats. The relative liver weight of rats fed Plumpynut was not significantly different (p >0.05) from those of animals fed PASLoc diets (MaLait and MiSoj). It was measured 3.00 ± 0.27 for the relative weight of the liver of the rats which had consumed the Plumpynut (Table 4).

Kidneys: According to the results reported in table 4, the relative weight of the kidneys of malnourished rats with Anagobaka was 0.43 ± 0.03. Higher values were obtained in groups of animals that received Plumpynut and PASLoc diets. The values recorded were 0.66 ± 0.07 (Plumpynut), 0.47 ± 0.04 (MaLait), 0.49 ± 0.04 (MiSoj) and 0.50 ± 0.02 (MaSojAr). Statistical analysis, however, showed that the relative weight of the kidneys of rats fed Plumpynut was significantly higher ($p < 0.05$) than those of animals fed PASLoc diets.

Spleen: Relative spleen weight was 0.32 ± 0.1 for Anagobaka-malnourished rats versus 0.33 ± 0.03 in Plumpynut-fed rats (Table 4). In animals that consumed PASLoc diets, the relative weight of the spleen was 0.28 ± 0.02 (MaLait), 0.20 ± 0.06 (MiSoj) and 0.26 ± 0.09 (MaSojAr). Relative weight measured in rats fed Plumpynut was not significantly different at $p > 0.05$ from those in rats fed PASLoc diets (Malait and MaSojar).

Abdominal fat: Observations showed that there was no abdominal fat in the malnourished rats with the low protein food (Anagobaka). On the other hand, abdominal fat was observed in the animals having received PASLoc and Plumpynut diets (Table 4). Indeed, abdominal fat with a relative weight of 0.32 ± 0.03 (Plumpynut), 0.66 ± 0.1 (MaLait), 0.34 ± 0.01 (MiSoj) and 0.36 ± 0, 02 (MaSojAr) were observed. The results also showed that there was a significant difference at $p < 0.05$ between Plumpynut and PASLoc MaLait while there was no significant difference at $p > 0.05$ between Plumpynut and PASLoc MiSoj and MaSojAr.

4. Discussion

The rats consumed the different foods they received. The corresponding total dry matter (TDM/d) was 3-4 g/d. The PasLocs were generally well consumed. They were even consumed more than the benchmark Plumpynut diet. These diets were therefore well accepted by the rats. This good consumption would be due to the nutritional quality of the food (balanced food), the organoleptic character and the texture making PasLoc a qualitative food as some authors stipulate [9-11]. Although Anagobaka was well consumed like other diets, it caused weight loss in rats. The good consumption of Anagobaka, on the other hand, could be attributed to its texture, smell and organoleptic character.

Table 4. *Impacts of test diets on relative organ weights of rats.*

Organs	Anagobaka	Plumynut	PASLoc		
			MaLait	Misoj	MaSojAr
Heart	0.24 ± 0.01*	0.32 ± 0.05	0.32 ± 0.24	0.28 ± 0.02	0.29 ± 0.01
Liver	2.02 ± 0.15*	3.00 ± 0.27	3.26 ± 0.20	2.81 ± 0.22	2.44 ± 0.33*
Kidney	0.43 ± 0.03****	0.66 ± 0.07	0.47 ± 0.04****	0.49 ± 0.04****	0.50 ± 0.02****
Spleen	0.32 ± 0.01	0.33 ± 0.03	0.28 ± 0.02	0.20 ± 0.06	0.26 ± 0.09
Abdominal fat	0****	0.32 ± 0.03	0.66 ± 0.04****	0.36 ± 0.04	0.31 ± 0.01

PASLoc: Specialized food products based on local ingredients, MaLait: Corn-Milk, MiSoj: Millet-Soy, MaSojAr: Corn-Soy-Groundnut. **** $p < 0.00001$, * $p < 0.05$: *significant difference compared to Plumpynut.*

The weight loss of the rats caused by Anagobaka and estimated on average at -1.12 ± 0.5 g/d would be attributable to the low quantity of ingested proteins (0.08 ± 0 g). Indeed, Anagobaka is very low in protein (2.04%). The results recorded during the malnutrition phase with Anagobaka are similar to those of previous works [6, 12]. These authors used Anagobaka to cause malnutrition in laboratory rats.

The weight loss characteristic of protein-energy malnutrition is due to a decrease in fat mass and then in lean mass [13]. Weight loss is the most important parameter for diagnosing protein-energy malnutrition [14]. Weight loss in animals would result from a slowdown in muscle protein synthesis caused by insufficient protein intake and increased energy expenditure [15]. In all malnourished rats given the PASLoc diets, remarkable growth was observed with weight gains of 1.26 ± 0.56 g/d (MaLait), 1.35 ± 0.3 g/d (MiSoj) and 0.75 ± 0.43 g/d (MaSojAr). Even if the weight gains favored by the PASLoc diets were lower than that of Plumpynut (1.86 ± 0.6), they allowed an increase in weight of the rats after malnutrition. This weight gain would be due to the PASLoc. According to the PNN that formulated them, PASLocs are balanced foods with acceptable nutrient levels. These diets based on local products are, like the reference food Plumpynut, able to cause weight gain in malnourished animals. PASLocs diets are capable of causing nutritional rehabilitation in the same way as the reference diet. And because of this, PASLoc could help reduce malnutrition.

The feed efficiency ratio (FER) estimated at 0.34 ± 0.1 (MaLait), 0.36 ± 0.08 (MiSoj) and 0.26 ± 0.5 (MaSojAr) and the protein efficiency ratio (PER) evaluated at 2.62 ± 0.6 (MaLait), 2.75 ± 0.59 (MiSoj) and 1.44 ± 0.60 (MaSojAr) clearly reflect the quality of these diets. Indeed, these values testify to the good assimilation and good yield of PASLoc diets studied. The PER calculated were on the whole close to 2, a value reflecting the good quality of a food [16, 17]. The results on FER and PER obtained with PASLoc diets are similar to those of Zannou-Tchoko [18]. This author had obtained FER values equal to 0.34 ± 0.027 and 0.35 ± 0.032 and PER values of 2.62 ± 0.24 and 2.27 ± 0.24 in rats fed respectively with flours based on soy and cassava used to fight against malnutrition in children of weaning age.

Examination of the relative weight values showed that, except for the kidneys, there was no difference concerning the other biological structures studied (liver, heart, spleen and abdominal fat) in the rats which received the different nutritional rehabilitation regimes. Overall, there was no observed hypertrophy or atrophy during weight change of the organs involved in food digestion and absorption in

malnourished rats fed PASLoc diets compared to those of the Plumpynut reference diet. The PASLocs did not cause any major structural modification and detectable anomaly at the macroscopic level. These results are in line with the PNN which formulated these nutritional rehabilitation diets. For the PNN, PASLoc are balanced foods with good nutritional value.

The relative weight values of the organs studied are certainly lower than those of previous works [7]. These values, however, are not alarming. The absence of abdominal fat observed in rats fed Anagobaka is a consequence of malnutrition. This is explained by the imbalance between insufficient calorie and/or protein intake and the body's needs, which are often increased. This leads to weight loss.

5. Conclusion

The PASLoc, used in the event of a stock shortage of conventional therapeutic foods, including Plumpynut, made it possible to obtain satisfactory results in terms of consumption parameters, rat growth and the biometric study of the organs regulating nutrition. However, in order to consolidate their use for nutritional rehabilitation, additional works must be carried out. This work will focus on the haematological and blood biochemical parameters, on the histology and histopathology of the organs of animals fed with these diets and also on the optimization of the nutritional performance of PASLoc by incorporating micronutrients.

Abbreviations

PNN: National Nutrition Program
PASLoc: Foods based on local products
PER: Protein efficiency ratio
FER: Feed efficiency ratio
MaLait: Corn-Milk
MiSoj: Millet-Soy
MaSojAr: Corn-Soy-Groundnut
RUTF: Ready-to-Use Therapeutic Nutritious Foods
MSHP: Ministry of Health and Public Hygiene/Ivory Coast
WG: weight gain
DMI: Total dry matter ingested
TIP: Total protein ingested

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