Content available at: https://www.ipinnovative.com/open-access-journals

International Journal of Clinical Biochemistry and Research

Journal homepage: https://www.ijcbr.in/

Original Research Article

Metabolic and histopathological impact of local resource food consumption on blood markers in rats previously subjected to moderate acute malnutrition

Natia Joseph Kouadio[1]*, Ange Laetitia Jocelyne Kouadio[1], Carine Ebah Assoumou[1], Aboutou Severin Kouassi Kra[1]

[1] *Felix Houphouet-Boigny University of Abidjan, Abidjan, Côte d'Ivoire*

ARTICLE INFO

Article history:
Received 22-08-2024
Accepted 27-09-2024
Available online 18-10-2024

Keywords:
Infant malnutrition
Infant diets
Albumins
CReactive proteins
Nutritional rehabilitation
Therapeutic diet

ABSTRACT

Background: Malnutrition is caused by dietary and nutritional imbalances that can have an impact on blood parameters and vital organs. The use of local agricultural resources for adapted diets appears to be an effective solution to post-weaning infant malnutrition. The aim of this study was to evaluate the effectiveness of four newborn diets made from local agricultural resources in improving biochemical, hematological, and histological parameters in rats that had previously experienced mild acute malnutrition.
Materials and Methods: A total of 42 juvenile male rats, with an average age of 80 ±5 days and an average weight of 103.46 ± 5.10 g, were divided into six groups. Each group consisted of seven rats, with two control groups (LTC and LSA) and four experimental groups (LAR1A, LAR1B, LAR2A, and LAR2B). The experiment had three distinct phases: an initial adaptation period lasting 5 days, followed by a phase of inducing malnutrition lasting 19 days, and finally a phase of nutritional rehabilitation lasting 14 days. At the conclusion of the malnutrition induction and nutritional rehabilitation phases, blood samples were collected and used to evaluate biochemical and haematological markers. Kidneys and liver were removed for histological analysis.
Results: The findings revealed that the period of inducing malnutrition had a detrimental impact on several parameters, such as urea, triglyceride, total protein, C-reactive protein, albumin, ALAT, ASAT, WBC, hemoglobin, hematocrit, and MCV. Nevertheless, the ingestion of LAR diet derived from indigenous ingredients successfully returned all of these blood markers to the required levels for rats. Furthermore, histological examinations demonstrated that there were no kidney or liver abnormalities at the end of the trial.
Conclusions: The LAR diets have rehabilitative effects on the biochemical and haematological parameters of rats. This suggests that these diets can be used therapeutically to treat moderate acute malnutrition and meet the nutritional needs of children aged 6 to 36 months.

1. Introduction

Protein-energy malnutrition (PEM) is a major public health issue that affects a large number of children between the ages of 6 and 36 months worldwide, with a particularly high occurrence in Africa (D'Souza et al., 2022).[1] Over the last five years, the incidence of PEM in sub-Saharan Africa has increased significantly, rising from 7.60% to 9.20% between 2018 and 2022 (FAOSTAT, 2020).[2] This syndrome is typically characterized by an insufficient intake of calories and/or protein. Severe cases of PEM cause significant changes in the biochemistry and structure of offspring, disrupting biological functioning and increasing the likelihood of developing numerous diseases (Tuca et al., 2013).[3] Protein-energy malnutrition in clinical practice

* *Corresponding author*.
E-mail address: jonatia2006@yahoo.fr (N. J. Kouadio).

https://doi.org/10.18231/j.ijcbr.2024.028

can be categorized into three types: marasmus, which is characterized by a deficiency in calories; kwashiorkor, which is characterized by a primary deficit in protein; and a combined form known as marasmus-kwashiorkor (Ibrahim et al., 2017).[4]

In 1996, UNICEF, the World Health Organization (WHO), and the World Bank joined together to create ready-to-use therapeutic diets specifically designed for children experiencing moderate and severe acute malnutrition. These products have been proven to be very effective in promoting the nutritional recovery of children affected by these types of malnutrition. They are widely used in the nutritional rehabilitation of children in the community and in health facilities, following the guidelines for the community-based management of acute malnutrition. Ready-to-use therapeutic diets are characterized by their immediate consumption, eliminating the need for water preparation, their palatable taste, and their extended shelf life of up to two years (Bazzano et al., 2017).[5] However, there is currently a significant shortage of readily available, ready-to-use therapeutic diets. Although efforts have been made to boost worldwide production, resulting in a 37% increase, and the number of suppliers has also expanded, the global supply of these items is still inadequate to satisfy the rising demand in 2022 (UNICEF, 2023).[6]

In order to tackle these different scarcities, the government of Côte d'Ivoire has developed a number of policies, such as providing outpatient nutritional treatment and adopting a national nutrition strategy in June 2015. Additionally, nutritional rehabilitation clinics, in collaboration with Ivorian research institutions and their partners, have produced complex diets using local ingredients such as cereals, seafood, and oilseeds. These facilities are managed by the Ministry of Health and Public Hygiene. The research done by Kra et al. (2022)[7] and Assoumou et al. (2022)[8] has shown the nutritional characteristics of the diets and porridges derived from them. These findings suggest that consuming these diets might be a successful strategy for addressing undernourishment and malnutrition. The prevalence of these issues in Côte d'Ivoire was expected to be 7.70% in 2022, according to FAOSTAT, (2020).

However, in order to accurately evaluate the capacity to restore the health of patients experiencing mild acute malnutrition, it is crucial to examine blood and histology parameters. Malnutrition and periods of dietary restriction have a significant impact on blood biochemical parameters as well as haematological and histological constants, as demonstrated by studies conducted by Leité et al. (2011) and Estrela et al. (2014).[9,10] A review of these diets will allow for a thorough evaluation of their effectiveness in rehabilitating people with mild acute malnutrition. The aim of this study is to address the issue of mild acute malnutrition in Côte d'Ivoire. The study aims to evaluate the ability of these diets to restore biochemical and hematological parameters in rats who have experienced mild acute malnutrition. Additionally, it will investigate the effects of consuming these diets on histological markers. The methodology will involve observing a disruption in blood parameters after inducing moderate acute malnutrition in young rats, and then confirming the restoration of these blood parameters (biochemical and hematological), as well as the tissue condition of specific vital organs (kidneys and liver).

2. Material and Methods

2.1. Animal batches and corresponding diets

A total of 42 male rats (Rattus norvegicus) of the Wistar strain, aged 80 ±5 days with an average weight of 103.46 ±5.10 g, were obtained from the vivarium of the Ecole Normale Supérieur (Abidjan, Côte d'Ivoire) and utilized in this study. The animals were maintained in individual metal cages under a 12-hour photoperiod and an average temperature of 25 ± 2 °C in the biological research laboratory of the aforementioned institution (Abidjan, Côte d'Ivoire). Six experimental batches of seven rats each were established: a control batch (LTC), an undernourished batch (LSA), and four batches each fed a diet composed of the national nutrition program, designated LAR1A, LAR1B, LAR2A, and LAR2B, respectively. The control group received a diet containing 16% casein as a protein source, whereas the undernourished group received a diet containing 4% protein. The control and undernourished diets were isocaloric and formulated according to the method described by Borelli et al. (2007).[11] Supplementary material 1 provides the composition of the control diets. Rats LAR1A, LAR1B, LAR2A, and LAR2B were fed different diets that were made from flours that contained soybean grains (Glycine max), rice grains (Oriza sativa), millet grains (Panicum miliaceum), maize grains (Zea mays), peanut grains (Arachis hypogea L.), brown sugar, vitamins A-rich palm oil, and fish meal (Clupea harengus). The biochemical composition of the various foods included in the national nutrition program is provided in supplementary material 2.

2.2. Experimental process

The experimentation was conducted over a period of 39 days, divided into two phases. The first phase, known as the adaptation phase, was used to help the experimental subjects adjust to the experimental conditions. The second phase, designated the assessment phase, was employed to assess the nutritional impact on biochemical, hematological, and histological constants. In the adaptation phase, all rats were fed 50 g of the control diet (LTC) for five days to enable them to overcome the stress and reach the same physiological stage. The assessment phase encompassed

two stages: a stage of malnutrition induction and a stage of nutritional rehabilitation. According to Borelli et al. (2007)[11] method, rats were malnourished by receiving 50 g of low-protein (4%) feed (LSA) every day for 19 days. All rats, with the exception of the LTC control batch, were fed this diet. At the conclusion of this phase, all rats in each batch exhibited a 20% reduction in body weight, in accordance with the recommendations of Borelli et al. (2007).[11] The nutritional rehabilitation phase entailed administering 50 g of the corresponding diet to the four LARd batches each morning between 8:00 and 9:00 a.m. The food scraps were recovered the following day, prior to any further distribution for 14 days. The control and undernourished batches continued to ingest their respective diets until the conclusion of the experiment.

2.3. Impact assessment on biochemical and haematological parameters

After completing the period of inducing starvation, three rats from each experimental group were sedated with urethane ether and subsequently euthanized. After the jugular veins were separated, a sample of whole blood was obtained. After completing the phase of nutritional rehabilitation, the surviving rats from each group were euthanized. Whole blood was obtained using hemolysis tubes containing an anticoagulant (EDTA) to investigate blood biochemical parameters. Hematological parameters were evaluated using violet tubes. The blood tubes were stored in a refrigerated container filled with ice. Afterwards, the blood samples were delivered to the laboratory of the Institut Pasteur de Côte d'Ivoire for automated examination.

2.4. Impact assessment on histological parameters

After sacrificing the animals, the kidneys and livers of each rat were removed using forceps and then stored in a 10% (V/V) formaldehyde solution for 48 hours. They were later rinsed with 70% ethanol. Afterwards, the tissues were put in compact metal containers and stirred using a magnetic stirrer. Subsequently, they were dehydrated using a sequence of alcohols, varying in concentration from 70% to 100%. Ultimately, they were immersed in kerosene using an enrober. Afterwards, the kerosene blocks were cut into sections using an advanced rotating microtome, placed on glass slides, and left to cure overnight. After being stained with hematoxylin and eosin (H&E), the slides were analyzed using a light microscope (Corrin, 1981).[12] The stained sections were analyzed using a Leica research microscope (DM 750) equipped with a digital camera (Leica ICC 50). Digital photomicrographs of the stained sections were obtained.

2.5. Statistical analysis

The values are expressed as means ± standard deviation. The data were analyzed using the XLSATAT V.2019.2.2 software. The figures were obtained using the Windows 10 Excel software. A Student's t-test for two independent samples was conducted at the conclusion of the induction period to assess the differences between the LTC control batch and the undernourished rat batch (LSA). Additionally, a Duncan test was conducted at the end of the nutritional rehabilitation phase to identify statistically significant differences. The overall significance level was set at a p-value of less than 0.05 for all tests.

3. Results

3.1. Impact of LAR diets on rat renal parameters

Table 1 displays the effects of several diets on rats' renal parameters after induction of malnutrition and nutritional rehabilitation. The malnutrition induction diet intake (LSA) had a significant effect ($P < 0.05$) on serum urea levels in LSA compared to LTC rats in both stages. The rats who were given the LSA diet had greater levels of urea (0.40 ±0.00 g/L) compared to the rats that were fed the LTC diet (0.22 ±0.03 g/L) and the LAR feed (0.12 ±0.02, 0.17 ±0.04, 0.14 ±0.02, and 0.21 ±0.09). There was no significant effect ($P > 0.05$) on the creatinine levels when compared to the LTC control diet.

Table 1: Rat renal parameters after malnutrition induction and nutritional rehabilitation

Phases	Batch of rats	Urea (g/L)	Creatinine (g/L)
Inducing malnutrition	LSA	0.40 ± 0.00^a	4.50 ± 0.50^a
	LTC	0.22 ± 0.03^b	5.00 ± 0.50^a
Nutritional rehabilitation	LAR1A	0.12 ± 0.02^b	4.00 ± 0.00^a
	LAR1B	0.17 ± 0.04^b	4.00 ± 0.00^a
	LAR2A	0.14 ± 0.02^b	4.00 ± 1.00^a
	LAR2B	0.21 ± 0.09^b	5.00 ± 1.00^a
	LTC	0.21 ± 0.08^b	4.00 ± 0.00^a
	LSA	0.39 ± 0.14^a	4.50 ± 0.50^a

In the same column, values marked with superscript letters (a, b, c, and d) are found to be significantly different at the 5% threshold.

3.2. Impact of LAR diet on rat energy and lipid metabolism

Figure 1 depicts the energy metabolism parameters of rats exposed to various diets at the end of the malnutrition induction and nutritional rehabilitation phases. Using Student's t-test, the levels of glucose and triglycerides in the blood of rats in the LSA batch were significantly ($P < 0.05$) different at the end of the malnutrition induction phase compared to the LTC batch, which had high levels. The blood glucose levels for the LSA and LTC batches were

0.80 g/L and 1.30 g/L, respectively. The triglyceride levels were 0.43 g/L and 0.70 g/L for the LSA and LTC rat batches, respectively. In contrast, the LDL/HDL ratio and serum total cholesterol levels (0.42 g/L to 0.49 g/L) of the two groups of LSA and LTC rats did not differ statistically significantly ($P > 0.05$).

There was a big difference ($P < 0.05$) in the levels of glucose, total cholesterol, triglycerides, and LDL/HDL in the blood of rats from the LTC and LSA batches compared to those fed the different LAR diets after the nutritional rehabilitation period was over. Rats fed the LAR1A, 1B, and 2A diets had statistically lower serum glucose levels than those fed the LTC and LSA control diets. The serum glucose levels were 0.85 g/L, 0.76 g/L, 0.87 g/L, 1.05 g/L, 1.40 g/L, and 1.10 g/L, respectively, for rats fed the LAR1A, LAR1B, LAR2A, LAR2B, LTC, and LSA diets. Rats that were fed the LAR1A (0.88 g/L), LAR2A (0.77 g/L), and LAR2B (0.74 g/L) diets had serum triglyceride levels that were statistically different from rats that were fed the LTC diet (1.55 g/L) but the same as rats that were fed the LSA diet (0.76 g/L). The LDL/HDL ratios for the rat batches fed the LAR1A and LAR1B diets were found to be below to 1, with values ranging from 0.19 to 0.39, respectively.

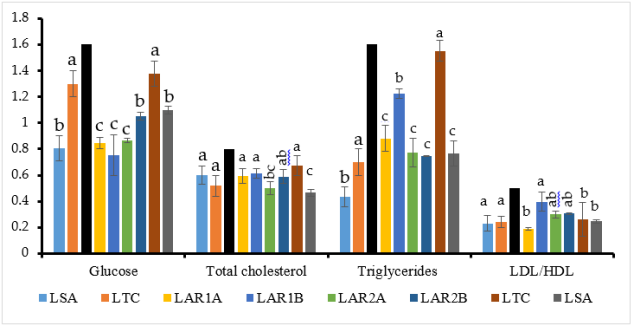

Figure 1: Rat energy and lipid metabolism during starvation and nutritional restoration (Histograms marked with different superscript letters (a, b, c, and d) are significantly different at threshold ($P < 0.05$)).

3.3. Impact of LAR diet on rat liver function

Table 2 illustrates the effect of LAR diet consumption on markers related to rat liver function during malnutrition induction and subsequent nutritional rehabilitation. A statistical analysis showed that making rats malnourished had a statistically significant effect ($P < 0.05$) on the levels of total protein, albumin, and conjugated bilirubin in their blood. This happened in both LSA and LTC rats. Rats fed the LTC diet had higher levels of these parameters than those fed the LSA diet. However, LSA rats' low levels of total protein, albumin, and conjugated bilirubin were corrected during the nutritional rehabilitation phase by consuming LAR diets. Statistical analysis demonstrated that serum total bilirubin levels were not affected by malnutrition induction in LSA and LTC rats. The statistical analysis revealed a significant impact on serum levels of C-reactive protein, ASAT, and ALTL, which differed between batches during the malnutrition induction and nutritional rehabilitation phases. However, the levels of C-reactive protein, aspartate aminotransferase (ASAT), and alanine aminotransferase (ALTL) were significantly lower ($P < 0.05$) than in rats from the LSA and LTC batches.

3.4. Impact of LAR diet on rat mineral metabolism

Figure 2 illustrates the serum mineral levels of rats at the end of both the malnutrition induction and nutritional restoration stages. The student's t-test reveals that there is no statistically significant difference in blood phosphorus, iron, and calcium levels between LSA and LTC rats, except for serum magnesium levels at the end of the malnutrition induction period.

The statistical analysis showed significant variation in the serum mineral levels of rats at the end of the nutritional rehabilitation phase. The calcium levels in rats given LAR diets were statistically similar to those in the LTC control diet and statistically distinct from those in the LSA batch. In terms of blood phosphorus levels, the LAR1A, LAR2A, and LAR2B rat batches showed no significant difference compared to the control diet, except for the LAR 1B rat batch. The magnesium levels in the blood of the LAR1A, LAR1B, LAR2A, and LAR2B rat groups were found to be statistically similar to those of the LSA-fed rats that had been starved. However, these levels were found to be statistically distinct from the control rat batch that was fed the LTC diet. Statistical analysis indicated a substantial disparity in iron levels across different groups of rats. Nevertheless, the LAR1A and LAR1B rats had increased iron levels, whereas the LAR2A, LAR2B, and LTC rat batches showed decreased iron levels.

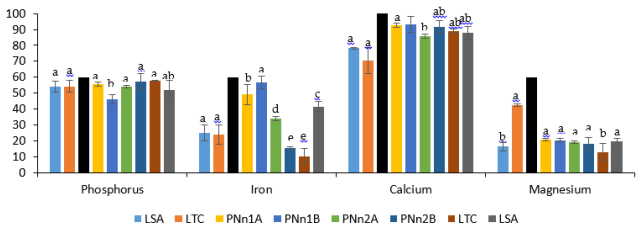

Figure 2: Rat serum mineral content after malnutrition induction and nutritional restoration. (Histograms marked with different superscript letters (a, b, c, and d) are significantly different at threshold ($P < 0.05$).

Table 2: Rat liver function markers after malnutrition induction and nutritional rehabilitation

Parameters	Inducing malnutrition		Nutritional rehabilitation					
	LSA	LTC	LAR1A	LAR1B	LAR2A	LAR2B	LTC	LSA
Total protein (g/L)	54.55 ±3.10^{b}	61.50 ±2.74^{a}	61.90 ±3.60^{a}	62.60 ±0.70^{a}	58.95 ±2.25^{a}	56.55 ±5.05^{a}	58.4 ±3.10^{a}	50.20 ±2.90^{b}
CRP (mg/L)	0.24 ±0.03a	0.15 ±0.02^{b}	0.18 ±0.06^{bc}	0.19 ±0.01^{bc}	0.20 ±0.03^{ab}	0.15 ±0.01^{c}	0.18 ±0.02^{bc}	0.28 ±0.03^{a}
Albumin (g/L)	27.09 ±4.91^{b}	31.76 ±11.42^{a}	30.65 ±2.99^{ab}	34.23 ±0.85^{a}	33.26 ±2.85^{ab}	31.70 ±1.35^{ab}	34.34 ±3.86^{a}	28.97 ±2.28^{b}
ALTL (UI/L)	48.00 ±1.40^{b}	63.00 ±3.00^{a}	31.00 ±2.50^{c}	29.10 ±8.70^{c}	38.40 ±2.20^{b}	21.90 ±2.90^{d}	37.45 ±3.25^{b}	118.75 ±5.15^{a}
AST (IU/L)	224.80 ±0.90^{a}	209.20 ±6.32^{b}	201.40 ±19.70^{ab}	174.50 ±44.60^{b}	173.95 ±7.15^{b}	156.75 ±26.15^{b}	204.35 ±11.55^{a}	215.35 ±8.25^{a}
Conjugated bilirubin (mg/dL)	0.01 ±0.01^{b}	0.05 ±0.00^{a}	0.26 ±0.28^{a}	0.10 ±0.07^{a}	0.30 ±0.36^{a}	0.16 ±0.10^{a}	0.22 ±0.03^{a}	0.05 ±0.08^{a}
Total bilirubin (mg/dL)	0.98 ±0.56^{a}	0.77 ±0.34^{a}	1.04 ±0.47^{a}	1.03 ±0.10^{a}	0.53 ±0.09^{b}	1.03 ±0.44^{a}	1.06 ±0.46^{a}	1.49 ±0.77^{a}

Superscript values (a, b, c, and d) are considerably different at 5% on the same line.

3.5. Impact of LAR diet on rat hematological parameters

Table 3 shows the rats' hematological constants at the end of the malnutrition induction and nutritional rehabilitation phases. When LSA rats were made to be malnourished, there was a statistically significant difference ($P < 0.05$) between them and LTC rats in the following parameters: blood platelets, lymphocytes, monocytes, eosinophils, basophils, and the number of white blood cells (WBCs). But there wasn't a big difference ($P > 0.05$) between the LSA and LTC rat batches in the number of red blood cells (RBC), mean corpuscular hemoglobin concentration (MCHC), neutrophils, erythroblasts, or immature granulocytes (IMG).

After the nutritional rehabilitation period ended, statistical analysis revealed that rats eating LAR diets had different effects on hemodynamic variables compared to rats eating LSA and LTC control diets. The amounts of white blood cells (WBC), red blood cells (RBC), and lymphocytes in the groups of rats that were fed LARd1A, LAR1B, LAR2A, and LAR2B were much higher than in the LSA group and much lower than in the LTC group. The results also show that the amounts of hemoglobin and hematocrit in the rats that were fed LARd1A, LAR1B, LAR2A, and LAR2B diets are statistically similar to those in the LSA rats that were not getting enough food. The rats in the LTC control group had the highest amounts of mean corpuscular hemoglobin concentration (MCHC), blood platelets, neutrophils, lymphocytes, and monocytes. These were followed by the rats that ate the different LAR diets. It is noteworthy that rats fed the malnutrition induction diet (LSA) exhibited the highest levels of neutrophils, monocytes, basophils, and immunoglobulin compared to the other batches of rats.

3.6. Impact of LAR diet on rat liver and kidneys after nutritional rehabilitation

Figure 3 shows histologic sections of the liver (Figure 3) and kidney (Figure 4) from rats fed the different diets at the end of the dietary rehabilitation. In the liver, analysis of sections from the different experimental batches shows the absence of abnormalities in the shape and size of hepatocytes (Ch). The trabeculae (T) and the centrolobular vein (Vc) are intact and also show no abnormalities. As for the condition of the kidneys, cross-sectional analysis showed no abnormalities after consumption of the different diets. X100 magnification in eosin shows that the urinary space (Eu), glomeruli (Gl), distal convoluted tubule (Tcd), and proximal convoluted tubule (Tcp) are intact.

4. Discussion

The capacity for rehabilitation of the various local diets was evaluated by examining parameters regulating renal and hepatic function, energy, lipid and mineral metabolism, and hematological parameters.

At the renal level, the study's results demonstrated that the consumption of LAR diets at the end of the nutritional rehabilitation period facilitated the normalization of serum urea levels, bringing them within the range recommended by Giknis and Clifford (2006),[13] between 0.13 and 0.16 g/L. This normalization of urea levels to recommended levels represented a significant health benefit for the young rats, given that urea and creatinine are the main metabolites regulated by the kidneys. Concentrations exceeding the recommended values may indicate the presence of abnormalities, such as protein malnutrition (Yoo et al., 2018).[14] Consequently, these findings underscore the safety of LAR diets and suggest that their consumption

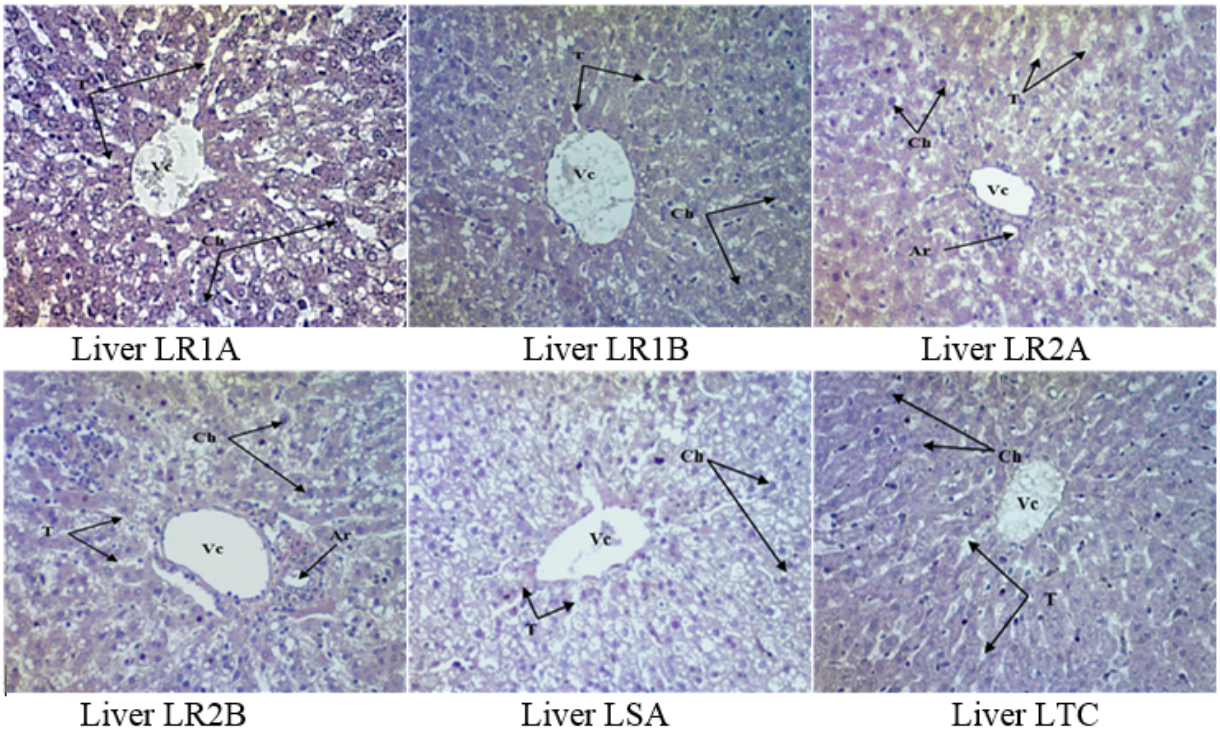

Figure 3: Histological sections of rat livers at the end of nutritional rehabilitation. Staining: Haematoxylin-Eosin; magnification: x100; Ch: Liver cell, T: Trabeculae, Vc: Centrilobular vein

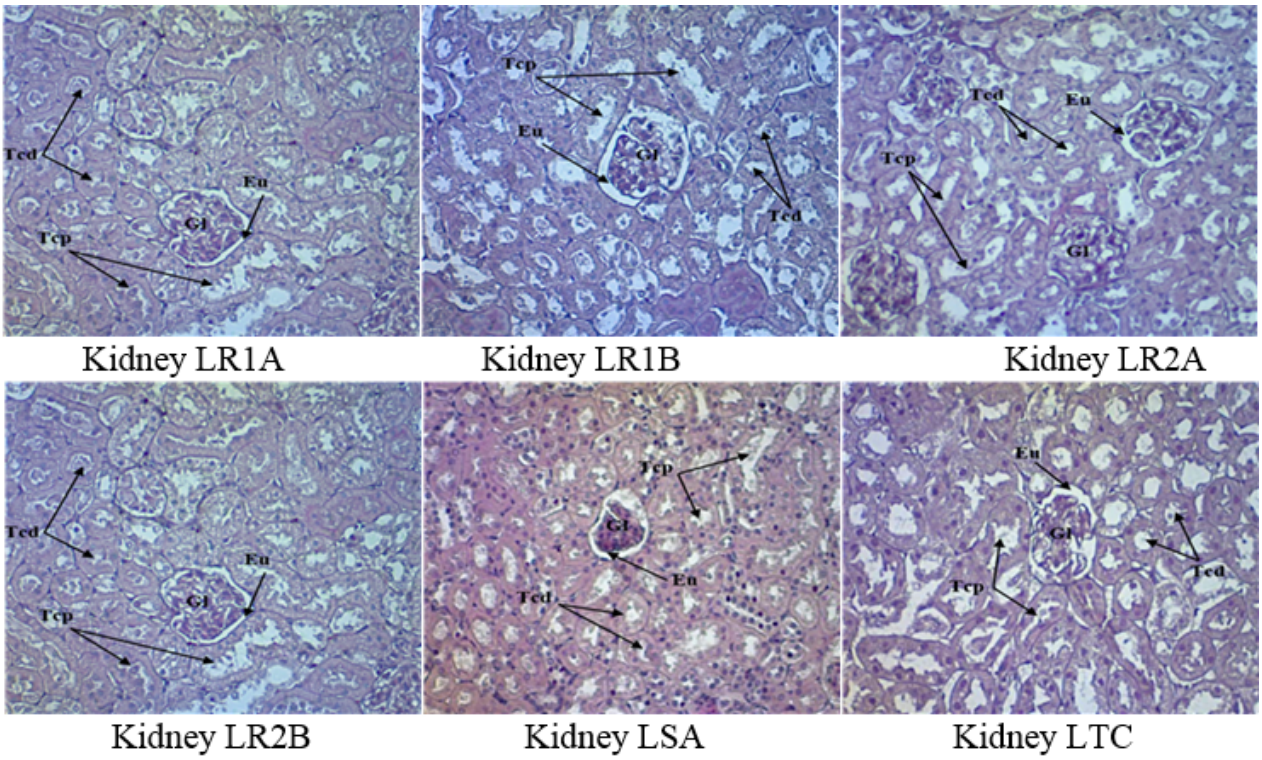

Figure 4: Histological sections of rat kidneys at the end of nutritional rehabilitation; Staining: Haematoxylin-Eosin; Magnification: x100; Eu: Urinary space, Gl: Glomerulus, Tcd: Distal convoluted tubule, Tcp: Proximal convoluted tubule.

Table 3: Rat liver function markers after malnutrition induction and nutritional rehabilitation

Parameters	Inducing malnutrition		Nutritional rehabilitation					
	LSA	LTC	LAR1A	LAR1B	LAR2A	LAR2B	LTC	LSA
WBC	16.97 ±1.46^{b}	25.96 ±10.63^{a}	15.42 ±3.85^{b}	17.03 ±5.52^{bc}	19.11 ±1.97^{b}	19.44 ±2.82^{b}	10.04 ±0.48^{c}	29.07 ±3.66^{a}
RBC	7.68 ±0.63^{a}	7.87 ±0.78^{a}	8.69 ±0.33^{ab}	7.98 ±0.64^{b}	8.18 ±0.45^{ab}	7.97 ±0.11^{b}	5.64 ±0.42^{c}	8.99 ±0.77^{a}
Hemoglobin	12.30 ±0.17^{b}	11.87 ±0.82^{a}	11.47 ±0.58^{b}	11.13 ±0.51^{b}	11.43 ±0.51^{b}	10.25 ±0.95^{b}	11.23 ±0.70^{b}	13.00 ±0.80^{a}
Hematocrit	38.37 ±0.42^{b}	42.70 ±0.52^{a}	41.50 ±0.30^{ab}	38.30 ±3.71^{b}	38.65 ±2.07^{b}	38.52 ±2.34^{b}	37.83 ±0.31^{b}	44.45 ±2.55^{a}
MCV	51.95 ±0.65^{b}	54.30 ±1.15^{a}	54.10 ±2.70^{ab}	54.90 ±0.70^{a}	53.90 ±0.86^{ab}	52.30 ±2.20^{abc}	51.40 ±0.70^{bc}	49.60 ±1.40^{c}
MCHCT	14.38 ±0.45^{b}	16.80 ±0.79^{a}	15.35 ±0.65^{a}	15.45 ±0.05^{a}	15.45 ±0.13^{a}	14.95 ±0.75^{a}	13.50 ±0.05^{b}	15.45 ±0.40^{a}
MCHC	28.52 ±0.29^{a}	30.90 ±0.22^{b}	28.40 ±0.20^{bc}	28.20 ±0.33^{c}	28.75 ±0.31^{b}	28.65 ±0.25^{bc}	28.58 ±0.30^{bc}	30.10 ±0.45^{a}
Blood platelets	590.50 ±38.50^{b}	928.67 ±14.34^{a}	698.50 ±4.50^{b}	412.00 ±78.00^{d}	473.50 ±38.50^{cd}	475.00 ±50.00^{cd}	544.00 ±73.00^{c}	828.50 ±25.50^{a}
Neutrophils	2.08 ±0.83^{a}	2.08 ±0.72^{a}	1.75 ±0.83^{ab}	1.14 ±0.36^{b}	1.76 ±0.33^{ab}	2.45 ±0.46^{ab}	4.78 ±3.54^{a}	4.41 ±1.7^{a}
Lymphocytes	14.16 ±0.68^{b}	21.98 ±0.07^{a}	15.53 ±5.75^{b}	8.58 ±4.88^{c}	17.01 ±4.10^{b}	15.88 ±5.49^{b}	11.41 ±2.01^{bc}	23.76 ±1.68^{a}
Monocytes	0.31 ±0.17^{b}	0.99 ±0.04^{a}	0.57 ±0.38^{a}	0.18 ±0.04^{b}	0.22 ±0.01^{b}	0.22 ±0.05^{b}	0.69 ±0.10^{a}	0.62 ±0.16^{a}
Eosinophils	0.40 ±0.21^{b}	0.87 ±0.20^{a}	0.33 ±0.21^{a}	0.13 ±0.06a^{b}	0.09 ±0.05^{b}	0.18 ±0.14^{ab}	0.07 ±0.00^{b}	0.24 ±0.05^{ab}
Basophils	0.03 ±0.01^{b}	0.04 ±0.00^{a}	0.03 ±0.02^{bc}	0.01 ±0.01^{c}	0.04 ±0.01^{bc}	0.03 ±0.01^{bc}	0.08 ±0.01^{a}	0.05 ±0.02^{b}
Erythroblasts	0.03 ±0.02^{a}	0.04 ±0.01^{a}	0.13 ±0.00^{a}	0.07 ±0.02^{b}	0.04 ±0.02^{bc}	0.06 ±0.05^{bc}	0.03 ±0.01^{c}	0.04 ±0.01^{bc}
IMG	0.02 ±0.01^{a}	0.02 ±0.01^{a}	0.02 ±0.01^{b}	0.02 ±0.02^{b}	0.03 ±0.00^{ab}	0.04 ±0.02^{ab}	0.08 ±0.07^{a}	0.06 ±0.02^{ab}

Superscript values (a, b, c, and d) are considerably different at 5% on the same line. MCV : mean corpuscular volume; MCHT : mean corpuscular hemoglobin content; MCHC : mean corpuscular hemoglobin concentration; WBC : White blood cells ; RBC Red blood cells; Hb : Haemoglobin, HTC : Hematocrit ; MCV : Mean corpuscular volume; MCH : Mean corpuscular hemoglobin content; MCHC : Mean corpuscular hemoglobin concentration; BPL : Blood platelets; IMG: Immunoglobulins.

could have a beneficial impact on renal function by regulating urea levels in children suffering from moderate acute malnutrition.

The liver plays a pivotal role as a central organ for the execution of various physiological processes that are essential for maintaining bodily homeostasis (Bessaguet & Desmoulière, 2021).[15] Some of these processes are the breakdown of xenobiotic compounds (Trefts et al., 2017),[16] the regulation of blood volume, the support of the immune system, the control of growth signaling pathways by endocrine hormones, and the homeostasis of lipids and cholesterol. Studies have shown that metabolic problems, such as changes in serum levels of total protein, albumin, C-reactive protein, ALTL, and AST, that were seen during the early stages of malnutrition were fixed by the end of the nutritional rehabilitation period after LAR diets were given.

The total protein concentrations are consistent with the recommendations of Johnson-Delaney (2008),[17] who suggest a range of 56 to 76 g/L. Indeed, serum protein levels are influenced by the protein and amino acid content of the foods consumed (Pennings et al., 2012; Yoshii et al., 2018).[18,19] It is well established that dietary proteins' nutritional quality influences serum protein biosynthesis, resulting in the production of biologically active molecules (Moughan et al., 2024).[20] Therefore, the higher serum total protein levels seen at the end of the nutritional rehabilitation period show that the rats that ate feed made from local products had a positive effect on their serum total protein levels after having moderate acute malnutrition.

The assessment of albuminemia proved to be of great value, as it is considered one of the main indicators of undernutrition under stable living conditions (Keller, 2019).[21] The results demonstrated a notable elevation in serum albumin concentrations in rats fed the various LAR diets in comparison to those on the malnutrition-inducing LSA diet. Nevertheless, only rats fed the LAR1B and LAR2A diets exhibited albumin concentrations within the range of 32 to 47 g/L, as recommended by Giknis and

Clifford, (2008). This discrepancy in serum albumin levels in comparison to the recommended values suggests a gradual recovery of these levels over time. The LAR1B and LAR2A diets demonstrated the capacity to rapidly restore albumin concentrations to recommended levels, indicating the potential benefit of their consumption in the management of moderate post-weaning acute undernutrition in Côte d'Ivoire.

C-reactive protein (CRP) is a biomarker of inflammation synthesized by the liver in response to acute phases of the organism. Its concentration increases significantly following bacterial infection, inflammation, or tissue damage (Naqvi et al., 2010; Bhattacharya & Munshi. 2023).[22,23] The results of the study revealed lower concentrations of this protein in the serum of rats fed diets composed of local products at the end of the nutritional rehabilitation phase compared with those fed the LSA diet during both phases. The low concentrations of C-reactive protein observed in the LAR groups indicate that the consumption of local produce-based diets not only attenuated the inflammatory phenomena and cellular damage induced by malnutrition, but also provided the molecules required for the biological recovery of these blood proteins.

In the liver, two enzymes, aspartic acid aminotransferase (ASAT) and alanine aminotransferase (ALAT), help move the amino group from L-aspartate and L-alanine to 2-oxoglutarate in a one-way process (Naqvi et al., 2010; Parivar et al., 2016).[23–25] High serum levels show that hepatic cells are being released through cytolysis (Parivar et al., 2016), and higher levels are linked to diseases like myocardial infarction and liver damage (Ndouyang & Himeda, 2018).[26] Researchers looked at liver enzyme activity in rats and found that rats fed local produce diets after nutritional rehabilitation had lower levels of ALTL and ASAT in their blood than rats fed the LSA diet, both during and after the rehabilitation period. The serum ALT levels in rats fed diets based on local ingredients were within the recommended range of 28 to 40 IU/L, as established by Giknis and Clifford (2008).[13] This compliance indicates that the liver regulates this enzyme's synthesis in a normal manner. However, the levels of aspartate aminotransferase (ASAT) in the blood of all the rat groups were higher than what Giknis and Clifford (2008)[13] suggested. For male rats 8 to 12 weeks old, the levels were between 87 and 114 international units per liter (IU/L). This increase in aspartate aminotransferase activity suggests that liver enzymes are leaking into plasma due to membrane impermeability (Naima & Zine, 2012).[27] However, histological sections revealed no evidence of dysfunction in these organs.

Bilirubin is a metabolite formed by the body's breakdown of hemoproteins, such as hemoglobins. Due to their toxic nature, these metabolites must be eliminated from the body if they accumulate (Sticova, 2013).[28] Our study's results demonstrated that serum concentrations in rats fed diets based on local produce were relatively close to the upper limit values recommended by Giknis and Clifford (2008), ranging from 0.1 to 1 mg/dL. Conversely, these relatively high concentrations could be beneficial for rats. Indeed, research by Hinds and Stec (2019)[29] and Creeden et al. (2021)[30] has demonstrated a positive correlation between high serum bilirubin levels and a reduced incidence of cardiovascular and metabolic diseases and their complications.

Energy parameters, including blood glucose, triglycerides, total cholesterol, HDL cholesterol, and LDL cholesterol, are employed to evaluate the influence of diverse dietary regimens on animal health. Serum glucose, an essential monosaccharide, is the primary source of energy for the human body, particularly the brain (Wang et al., 2023).[31] Accurate blood glucose measurement is a fundamental aspect of the screening, diagnosis, and monitoring of diabetes and related conditions (Wang et al., 2023).[31] Our study's findings indicate that rats fed the LTC diet had lower glucose levels than rats fed the LTC control diet. Moreover, these blood glucose values align with the recommendations set forth by Johnson-Delaney (2008),[17] who suggest a range of 0.5 to 1.40 g/L. Compliance with the standards indicates that consumption of LAR food had no negative effect on the rats' blood glucose levels. Rather, it met their energy requirements, demonstrating the metabolization of carbohydrate macromolecules (Kurtz et al., 2013).[32] When it comes to the blood glucose levels of rats in the LSA and LTC groups fed the control diets, compliance with the standards recommended by Johnson-Delaney (2008) can be attributed to the fact that these diets were formulated to provide the amount of calories needed to meet the rats' energy requirements.

The plasma lipid profile, which is shown by the levels of total cholesterol and triglycerides, was lower in rats that ate the different LAR diets than in rats that ate the LTC diet. Due to the fat content and lipid profiles of the rats' foods, this significant difference may exist. The total cholesterol levels observed in this study were consistent with the recommendations set forth by Johnson-Delaney (2008),[17] ranging from 0.40 to 1.30 g/L. Similarly, triglyceride levels in LAR1A, LARd2A, LARd2A, and LARd2B diets were within the Johnson-Delaney (2008)[17] guidelines, ranging from 0.26 to 1.45 g/L.

According to Lorente-Cebrian et al. (2013),[33] consuming these foods in accordance with dietary standards has been shown to reduce the risk of cardiovascular disease. Indeed, research conducted by Tomonori et al. (2007)[34] indicated a correlation between a high concentration of polyunsaturated fatty acids and an elevated risk of all-cause mortality. High-density lipoproteins (HDL) are recognized for their protective role against atherosclerosis and coronary heart disease, as Tedgui (2022)[35] has pointed out. In our study, the LDL/HDL ratio was consistently below 1 for all diets under

investigation, indicating elevated serum HDL cholesterol levels. The high concentration of HDL cholesterol confers an advantage on individuals consuming these foods, as the beneficial effects of these lipoproteins on the body have been well documented.

During the induction phase, rats fed the LSA diet had a lower serum magnesium concentration than rats fed various LAR diets during the rehabilitation period. This discrepancy can be attributed to the reduced magnesium intake observed in rats fed the LSA diet. A magnesium-deficient diet results in a rapid decrease in serum magnesium levels in rats, accompanied by alterations in its cellular distribution (Andrieux-Dumond and Le, 1974).[36] Calcium and magnesium are among the most abundant minerals in the body and play a crucial role in numerous physiological functions. As reported by Zoroddu et al. (2019),[37] magnesium is involved in over 300 biochemical reactions within the body. When magnesium intake is reduced in cases of undernutrition, magnesium deficiency can result in hypomagnesemia (Lwin & Mon, 2020).[38] Phosphorus and calcium also play a role in bone and dental health, contributing to their formation and maintenance (Boskey, 2006).[39] The findings of the study on the magnesium content of rats fed different LAR suggest that these products may be effective in restoring magnesium levels in malnourished rats.

A comprehensive analysis of blood parameters in rats during the induction and rehabilitation phases revealed a number of significant variations in parameters related to malnutrition, including leukocytes, hemoglobin, and lymphocytes. Less leukocytes, erythrocytes, hemoglobin, hematocrit, and lymphocytes were found in rats fed the LSA diet during the induction phase compared to rats fed the LAR diet during the rehabilitation phase. Nutrient deficiency, particularly protein deficiency, has been shown to disrupt numerous biological processes, including leukopoiesis. This reduction in leukopoiesis compromises the establishment of a rapid and effective immune response, leading to leukopenia. Leukopenia is characterized by a reduction in the number of leukocytes, as well as an alteration in the functions performed by these cells (Santos et al., 2017).[40] These findings corroborate previous research by De Morais et al. (2016),[41] which indicates that animals subjected to dietary restriction exhibit a state of immunodepression, rendering them more susceptible to infection and disease. Leukocytes play a pivotal role in the body's immune response to pathogens and antigens. Taking LAR1A, LAR1B, LAR2A, and LAR2B could help restore normal levels of different blood parameters that become off while rats are being used in experiments, without having any negative effects on blood constants. Furthermore, the restoration of leukocyte, erythrocyte, hemoglobin, hematocrit, and lymphocyte levels suggests that LAR diets have therapeutic properties that enable the body to combat anemia, inflammation, and microbial infections.

Biochemical and hematological tests were done on the young rats that were fed the different foods. The liver and kidneys were also studied histologically and histopathologically to see what effect the food had on these important organs.

No abnormalities were observed in the kidneys. Indeed, microscopic analysis indicates that the cells involved in kidney filtration are normal and are functioning as intended, purifying the blood by eliminating waste products from the body's functioning and maintaining the chemical balance of the blood (Leite et al., 2011; Estrela et al., 2014).[9,10] In the liver, microscopic observation revealed the presence of normal liver tissue architecture. This indicates that the consumption of LAR diets had no adverse effect on these organs.

5. Conclusions

The study's goal was to find out how well LAR diet could restore biochemical, hematological, and histological constants in rats that had been through moderate acute malnutrition. This was done to help combat the moderate-acute malnutrition that occurs in Côte d'Ivoire. The analysis results showed that consuming a LAR diet made from local products successfully restored all blood parameters that had been negatively affected by malnutrition induction. The rehabilitative properties of LAR diets on the biochemical and hematological parameters of the rats permit an understanding of the therapeutic impact of these diets in the fight against acute malnutrition in Côte d'Ivoire and the coverage of the nutritional needs in the blood compounds of children aged between 6 and 36 months. The absence of pathological changes in histological parameters indicates that consumption of LAR diets does not result in any adverse effects. It would therefore be of interest to produce these different LAR in the form of ready-to-eat foods, with the aim of facilitating their use by 36-month-old children in rural and urban communities.

6. Statement on the Welfare of Animals

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

7. Sources of Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

8. Conflicts of Interest:

The authors declare no conflict of interest concerning this study.

References

1. Souza VD, Madhura RJ, Shetty M, Varsha A, Chakraborty A, Kumar MB, et al. Protein malnutrition in BALB/c mice: An experimental model resembling clinical scenario. *J Health Alli Sci NU*. 2023;13(4):490–6.
2. 2.FAOSTAT. Food and Agricultural Organization, Agricultural Data. Food Security and Nutrition Domain. Rome, Italy; 2020. Available from: https://www.fao.org/faostat/fr/#data/FS.
3. Tuca A, Jimenez-Fonseca P, Gascón P. Clinical evaluation and optimal management of cancer cachexia. *Crit Rev Oncol Hematol*. 2013;88(3):625–36.
4. Ibrahim MK, Zambruni M, Melby CL, Melby PC. Impact of childhood malnutrition on host defense and infection. *Clin Microbiol Rev*. 2017;30(4):919–71.
5. Bazzano A, Potts K, Bazzano L, Mason J. The life course implications of ready to use therapeutic food for children in low-income countries. *Int J Environ Res Public Health*. 2017;14(4):403. doi:10.3390/ijerph14040403.
6. UNICEF. Ready-to-Use Therapeutic Food: Market and Supply Update, pdf (unicef.org). UNICEF Supply Division; 2023. Available from: https://www.unicef.org/supply/reports/ready-use-therapeutic-food-market-and-supply-update.
7. Kra KAS, Assoumou EC, Kouadio NJ, Kouadio AJL, Niamke S. Nutritional and Rheological Characterization of Four Porridges Prepared from Local Products in View to Rehabilitating Moderate Acute Malnutrition. *Am J Food Nutr*. 2023;10(3):65–71.
8. Assoumou EC, Kra KAS, Kouadio NJ, Kouadio AJL. Nutritional qualities and vitamin content of local flours used in nutritional rehabilitation centers in Côte d'Ivoire. *EAS J Nutr Food Sci*. 2022;4(5):146–55.
9. Leite SN, Jordão JAA, Andrade TAM, Masson3 DS, Frade MAC. Modelos experimentais de desnutrição e sua influência no trofismo cutâneo. *An Bras Dermatol*. 2011;86(4):681–8.
10. Estrela DCC, Lemes GC, Guimarães ATB, Malafaia G. Effects of short-term malnutrition in rats. *Scientia Plena*. 2014;10(7):1–14.
11. Borelli P, Blatt S, Pereira J, Maurino BBD, Tsujita M, de Souza AC, et al. Reduction of erythroid progenitors in protein-energy malnutrition. *Br J Nutr*. 2007;97(2):307–14.
12. Corrin B. Carleton's histological technique. *J Clin Pathol*. 1981;34(5):572. doi:10.1136/jcp.34.5.572-d.
13. Giknis ML, Clifford CB. Clinical Laboratory Parameters for Crl: CD (SD) Rats. Charles Rivers Laboratories,; 2006. p. 1–18.
14. Yoo SH, Hong JS, Yoo HB, Han TH, Jeong JH, Kim YY, et al. Influence of various levels of milk by-products in weaner diets on growth performance, blood urea nitrogen, diarrhea incidence, and pork quality of weaning to finishing pigs. *Asian-Aus J Animal Sci*. 2018;31(5):696–704.
15. Bessaguet F, Desmoulière A. Le foie. *Actualites Pharmaceutiques*. 2021;60(605):57–61.
16. Trefts E, Gannon M, Wasserman DH. The live. *Curr Biol: CB*. 2017;27(21):1147–51.
17. Johnson-Delaney CA. Exotic Companion Medicine Handbook For Veterinarians, Eastside Avian & Exotic Animal Medical. Eastside Avian & Exotic Animal Medical Center Kirkland; 2008.
18. Pennings B, Groen B, De Lange A, Gijsen AP, Zorenc AH, Senden JMG, et al. Amino acid absorption and subsequent muscle protein accretion following graded intakes of whey protein in elderly men. *Am J Physiol Endocrinol Metab*. 2012;302(8):992–9.
19. Yoshii N, Sato K, Ogasawara R, Nishimura Y, Shinohara Y, Fujita S, et al. Effect of mixed meal and leucine intake on plasma amino acid concentrations in young men. *Nutrients*. 2018;10(10):1543. doi:10.3390/nu10101543.
20. Moughan PJ, Fulgoni VL, Wolfe RR. The importance of dietary protein quality in mid- to high-income countries. *J Nutr*. 2024;154(3):804–14.
21. Keller U. Nutritional laboratory markers in malnutrition. *J Clin Med*. 2019;8(6):775. doi:10.3390/jcm8060775.
22. Bhattacharya S, Munshi C. Biological significance of C-reactive protein, the ancient acute phase functionary. *Front Immunol*. 2023;14:1238411. doi:10.3389/fimmu.2023.1238411.
23. Naqvi S, Samim M, Abdin MZ, Ahmad FJ, Prashant CK, Dinda A, et al. Concentration-dependent toxicity of iron oxide nanoparticles mediated by increased oxidative stress. *Int J Nanomedicine*. 2010;5:983–9. doi:10.2147/IJN.S13244.
24. Zachoval R. Transaminasen. *MMW Fortschritte der Medizin*. 2021;163(9):51. doi:10.1007/s15006-021-9808-8.
25. Parivar K, Malekvand FF, Bayat M, Alavian SM, Motavaf M. Evaluation of iron oxide nanoparticles toxicity on liver cells of BALB/c rats. *Iran Red Crescent Med J*. 2016;18(1):e28939. doi:10.5812/ircmj.28939.
26. Ndouyang CJ, Himeda RMM. Antinutriments et propriétés nutritionnelles in vivo de Cochlospermum tinctorium A. Rich. (Bixaceae) chez les jeunes rats (Rattus norvegicus L.). *Int J Biol Chem Sci*. 2018;12(2):884–901.
27. Naima L, Zine K. Combined protective effect of vitamins C and E on cadmium induced oxidative liver injury in rats. *Afr J Biotechnol*. 2012;11(93):16013–20.
28. Sticova E. New insights in bilirubin metabolism and their clinical implications. *World J Gastroenterol: WJG*. 2013;19(38):6398. doi:10.3748/wjg.v19.i38.6398.
29. Hinds TD, Stec DE. Bilirubin safeguards cardiorenal and metabolic diseases: A protective role in health. *Curr Hypertens Rep*. 2019;21(11):87. doi:10.1007/s11906-019-0994-z.
30. Creeden JF, Gordon DM, Stec DE, Jr TH. Bilirubin as a metabolic hormone: the physiological relevance of low levels. *Am J Physiol Endocrinol Metab*. 2021;320(2):191–207.
31. Wang Z, Du Y, Zhang Q, Yan J, Zhan M, Zhang P, et al. Development of a glucose reference material in human serum for clinical assay standardization. *J Pharm Biomed Anal*. 2023;228:115285. doi:10.1016/j.jpba.2023.115285.
32. Kurtz P, Claassen J, Schmidt JM, Helbok R, Hanafy KA, Presciutti M, et al. Reduced brain/serum glucose ratios predict cerebral metabolic distress and mortality after severe brain injury. *Neurocritical Care*. 2013;19(3):311–9.
33. Lorente-Cebrián S, Costa AGV, Navas-Carretero S, Zabala M, Martínez JA, Moreno-Aliaga MJ, et al. Role of omega-3 fatty acids in obesity, metabolic syndrome, and cardiovascular diseases: a review of the evidence. *J Physiol Biochem*. 2013;69(3):633–51.
34. Tomonori O, Hideo T, Naomi M, Takehito H, Takashi K, Yoshikuni K, et al. The relationship between serum total cholesterol and all-cause or cause-specific mortality in a 17.3-year study of a Japanese cohort. *Atherosclerosis*. 2007;190(1):216–23.
35. Tedgui A. Actualités dans la physiopathologie de l'athérosclérose. *Revue Francophone des Laboratoires*. 2022;(543):26–34. doi:10.1016/s1773-035x(22)00212-x.
36. Andrieux-Dumond C, Le V. La Carence En Magnésium Chez Les Rats Au Sevrage Et Ses Conséquences Intracellulaires. *Ann Nutr Alim*. 1974;28(6):549–67. Available from: http://www.jstor.org/stable/45122961.
37. Zoroddu MA, Aaseth J, Crisponi G, Medici S, Peana M, Nurchi VM. The essential metals for humans: a brief overview. *J Inorganic Biochem*. 2019;195:120–9. doi:10.1016/j.jinorgbio.2019.03.013.
38. Lwin ZT, Mon AW. A survivor from Extremely Severe Metabolic Acidosis. *G Med Sci*. 2020;1(2):16–9.
39. Boskey AL. Mineralization, structure, and function of bone. In: Dynamics of Bone and Cartilage Metabolism: Principles and Clinical Applications. vol. 65. Elsevier; 2006. p. 201–9.
40. Santos EW, Oliveira DC, Silva GB, Tsujita M, Beltran JO, Hastreiter A, et al. Hematological alterations in protein malnutrition. *Nutr Rev*. 2017;75(11):909–9.
41. De Morais N, Costa TD, Pedrosa ALF, De Castro M, De Albuquerque SDG, Pereira VRA. Effect of neonatal malnutrition on expression of nitric oxide synthase enzyme, production of free radicals and in vitro viability of alveolar macrophages infected with methicillin-sensitive and methicillin-resistant Staphylococcus aureus. *Eur J Nutr*. 2016;55(1):403–11.

Author biography

Natia Joseph Kouadio, Researcher https://orcid.org/0000-0002-9337-4495

Ange Laetitia Jocelyne Kouadio, PhD https://orcid.org/0000-0002-4135-3529

Carine Ebah Assoumou, PhD https://orcid.org/0000-0003-3652-7843

Aboutou Severin Kouassi Kra, Professor-Researcher https://orcid.org/0000-0002-9269-5465

Cite this article: Kouadio NJ, Kouadio ALJ, Assoumou CE, Kra ASK. Metabolic and histopathological impact of local resource food consumption on blood markers in rats previously subjected to moderate acute malnutrition. *Int J Clin Biochem Res* 2024;11(3):187-197.