Effect of PASLoc Meals on the Zootechnical and Nutritional Parameters of Young Wistar Rats Suffering from Induced Moderate Acute Malnutrition

.Abstract :

Aims: The objective of this study was to assess the efficacy of four food products manufactured from locally sourced ingredients in restoring Wistar rats afflicted with induced moderate acute malnutrition.

Study design: The rats used in this research were kept in separate metabolic cages with wire mesh flooring.

Place and Duration of Study: The study was conducted at the vivarium of the Ecole Normale Supérieure in Abidjan, Côte d'Ivoire, over a period of 38 days.

Methodology: 42 male rats, with an average age of 80 ±5 days and an average weight of 103.46 ±5.10 g, were randomly divided into six batches of seven rats each. Two control batches (MID and PCD) and four experimental batches (PASLoc1A, PASLoc1B, PASLoc2A, and PASLoc2B) were formed. The experiment consisted of an adaptation phase (5 days), a malnutrition induction phase (19 days), and a nutritional rehabilitation phase (14 days). Zootechnical and nutritional parameters were assessed at the end of the induction and nutritional rehabilitation phases.

Results: Malnourished animals experienced a body weight loss rate of -22.36 ±6.47%, leading to a decline in all zootechnical parameters, including weight gain (1.39 ±0.28 g/day), body length (0.95 ±0.64 cm), and feed efficiency coefficient (-24.98 ±5.47). The nutritional rehabilitation phase resulted in the correction of the dysfunctions observed during induction, especially in terms of body weight gain, with rates comprised between 20.84 ±5.03% and 34.59 ±3.33%. The minimum weight gain for the rats fed PASLoc diet was 1.66 ±0.54 g/d for Pasloc1B, respectively. PASLoc diets have a significant impact on nutritional value, with a minimum biological value of 94.04 ±0.25%.

Conclusion: The result of this study suggest that PASLoc2A and PASLoc2B possess a robust nutritional potential, and their consumption can effectively address issues of moderate acute malnutrition after weaning.

Keywords: Induced malnutrition, local specialized food products, moderate acute malnutrition, nutritional assessment, zootechnical parameters.

1. INTRODUCTION

Malnutrition is a pathological condition that is caused by a mismatch between the food intake and the needs of the body or by a metabolic disorder [1] [2]. It takes two main forms, which are undernutrition and overnutrition. According to the World Health Organization [3], women, adolescents, and especially children and infants in developing countries are most at risk. The most prevalent form, undernutrition, remains a pervasive global public health concern [4].

In Côte d'Ivoire, the national nutrition program indicates that the prevalence of three undernutrition indicators (i.e., acute malnutrition, chronic malnutrition, and underweight) is considered to be of concern [2]. Indeed, the findings of the 2021 Demographic and Health Survey indicate that the prevalence of acute malnutrition among children aged 0-59 months is 8%, including 2% with severe forms. Furthermore, the survey reveals that 23% of children under 5 suffer from chronic malnutrition, exhibiting notable regional variations. With regard to underweight children, the Goh-Djiboua and Savanes regions are noteworthy for having 25% and 20%, respectively, of their children affected [5].

In response to the prevalence of malnutrition, the government of Côte d'Ivoire has initiated a series of initiatives. Some of these interventions are systematized in intervention packages. These include, for example, outpatient nutritional care; the adoption of a national nutrition policy in June 2015; and the implementation of a multisectoral national nutrition plan in may 2016. This plan consists of the distribution of specialized food products, formulated by public research and the agrifood industry, known as "special food products for special dietary use" [2] [6]. Nevertheless, although the Programme National de Nutrition (PNN) has some funding for the supply of PAS, it is currently not possible to meet all demands [7]. The Ministry of Health and Public Hygiene, in collaboration with Ivorian research institutes and their partners, has developed compound flours called PASLoc, based on local products such as cereals, fish, and oilseeds, to address this situation [8]. However, researchers have not conducted any studies to evaluate the effectiveness of these food products in restoring individuals suffering from moderate acute malnutrition or determining the impact of meal PASLoc on the zootechnical and nutritional parameters of young wistar rats. An in vivo model using rats may be a suitable means of evaluating the ability of these food products to restore or not restore an individual with moderate acute malnutrition [9,10,11].

The overarching goal of this study is to address the issue of moderate acute malnutrition in Côte d'Ivoire by proposing novel food formulations based on locally sourced ingredients. The specific goal is to see how well four PASLoc feeds work at restoring zootechnical and nutritional constants in a group of young Wistar rats that had been through a model of moderate acute malnutrition. The methodology involved inducing moderate acute malnutrition in the young rats, rehabilitating them through the consumption of PASLoc feeds, and assessing their restoration by measuring growth parameters.

2. MATERIAL AND METHODS

2.1. Raw Materials for Local Infant Food Formulation

The grains utilized in the local flours in this study included millet (*Panicum miliaceum*), soybean (*Glycine max*), maize (*Zea mays*), rice (*Oriza sativa*), and groundnut (*Arachis hypogea L*.). We procured these grains from the Forum Market in Abidjan, Côte d'Ivoire. We procured ready-to-use products, including brown sugar and palm oil enriched with vitamin A, from a shopping mall in the Abidjan district. We purchased fish meal, specifically *Clupea harengus*, from the autonomous port of Abidjan (Côte d'Ivoire).

2.2. Processing raw materials

The various raw materials were processed into flours according to the protocol described by Kra et al. [12]. Millet, soybean, maize, rice, groundnut, and fish grains were processed into flours and then stored in glass bottles at room temperature (25 °C) in the laboratory for later use.

2.3 Infant Food Formulation

The various infant foods were formulated according to the recommendations of the Ivorian National Nutrition Program, established in 2015 [13]. Four types of food based on local products were formulated by combining the flours previously obtained [12]. Table 1 displays the nutritional composition of the PASLoc feeds formulated.

Table 1: Nutritional composition of PASLoc foods

Parameters	PASLoc1A	PASLoc1B	PASLoc2A	PASLoc2B
Dry Matter (%)	96.26	96.54	96.10	96.44
Ash (%)	1.50	2.10	2.60	1.80
Fats (%)	9.66	21.53	23.86	21.66

Proteins (%)	11.19	15.72	18.12	13.82
Carbohydrates (%)	73.90	57.18	51.50	59.15
Energy Value (Kcal/100g)	427.36	485.42	493.33	486.86
Iron (mg)	4.30	4.26	3.83	4.2
Zinc (mg)	1.06	1.13	0.76	0.86
Vitamin A (µg/100g)	17.80	15.70	78.63	11.07
Vitamin E (mg/100g)	15.63	30.36	28.13	30.46
Vitamin D (µg/100g)	0.39	0.23	0.11	0.48
Vitamin B1 (mg/100g)	0.56	0.50	0.59	0.46

2.4. Design of Control Diets

Two iso-caloric control diets (410.6 kcal/100 g) were established according to the method described by Borelli et al. [14]: a positive control diet (PCD) and a malnutrition induction diet (MID). The positive control diet (PCD) contains 20% casein as a protein source, while the malnutrition induction diet (MID) contains 4% of casein. Table 2 provides a list of ingredients used in these diets.

Table 2: Composition of control diets

Constituents	PCD (mg/g)	MID (mg/g)
Casein (85 % protéines)	0.200	0.040
Sucrose	0.100	0.100
Fibres	0.010	0.010
corn oil	0.080	0.080
Mineral blend	0.040	0.040
Vitamin blend	0.010	0.010
L-Méthionine	0.0015	0.0015
Bitartrate de choline	0.00025	0.0025
Corn starch	0.5565	0.7165

The diets are iso-caloric and provide 1716.3 kJ / 100 g (410.6 kcal / 100 g). PCD: Protein Control Diet; MID: Malnutrition Induction Diet. Source: [14].

2.5. Evaluation of the ability of PASLoc diets to rehabilitate previously malnourished rats

Nutritional rehabilitation was conducted in accordance with the methodology described by Kouadio et al. [15], using Wistar rats. This process was divided into two main phases: the phase of establishing rat cohorts and allocating them to metabolic cages, and the experimental phase, which consisted of three stages: adaptation, induction of moderate acute malnutrition, and nutritional rehabilitation.

For the experimental batch constitution phase, young rats of the Wistar strain (*Rattus norvegicus*) aged on average 80 ±5 days with an average weight of 103.46 ±5.10 g were utilized. The 42 rats were divided into six batches of seven rats each, based on their relatively uniform weight. This resulted in two control batches (positive and negative) and four test batches. The rats were placed in individual metabolic cages, the bottoms of which were lined with a smaller mesh to collect food scraps and separate feces from urine. The cages were equipped with food racks and water bottles. The metabolic cage was set up in a room maintained at a temperature of 28 ± 2 °C, a relative humidity ranging from 70 to 80%, and a light-dark cycle of 12 hours. The experimental phase was conducted as follows:

- An adaptation stage: All six batches of rats were fed only the protein control diet (PCD) (positive diet) along five days.

- A malnutrition inducing stage: One batch continued to be fed the positive diet (protein control diet: PCD), while the other five batches were fed the malnutrition inducing diet (MID) (negative controle) along 19 days. Rats were fed for Moderate acute malnutrition. Moderate acute malnutrition is said to be inducing when the rats have lost at least 20% of their body weight [14].
- A Nutritional rehabilitation stage: one batch continued to fed the protein control diet, one batch continued to fed the malnutrition inducing diet (MID), and four batches were each fed a PASLoc diet. The nutritional rehabilitation stage of the rats lasted 14 days.

During the adaptation, malnutrition induction, and nutritional rehabilitation stages, each diet was diluted in distilled water at a ratio of 1:2 (w/v) and then cooked for 10 minutes on a hot plate. The resulting mashed was then cooled and distributed to the racks at a rate of 50 grams. Each morning, 50 grams of prepared food in the form of mashed are distributed to the rats. The following day, any remaining food or waste is collected and weighed before being redistributed. Water was provided *ad libitum* via feeding bottles.

The young rats were weighed at the outset of the experiment and then at three-day intervals until the experiment's end. Over the final five days of the study, daily feed fractions, feces, and urine from each rat were collected and stored for the nitrogen balance study. The zootechnical parameters presented in Table 3 were evaluated at the beginning of the experiment, then at three-day intervals, and finally at the end of the experiment.

Table 3: Parameters for studying the nutritional impact of different diets

Parameters	Mathematical expressions
Rate of weight change (%)	RWC= (final weight - initial weight)/ final weight × 100
Weight gain (g/d)	WG = (final weight –initial weight) / Nombre de jours
Body length (cm)	BL = Longueur finale – Longueur initiale
Abdominal circumference (cm)	AC = Circonférence finale – Circonférence initiale
Body mass index	BMI = P/T^2
Dry matter intake (g/d)	DMI = ((QAS – QAR) × Taux de MS) /Nombre de jours
Feed efficiency coefficient	FEC = Gain de poids / QMSI
Total protein intake (g)	TPI = QMSI × % Protéine du régime
Apparent protein digestibility (%)	Da = [(I – F) / I] × 100
Real protein digestibility (%)	Dr = [(I – (F – Fsp) / I] × 100
Protein efficiency coefficient	PEC = GP / PTI
Protein retention	PR = I – (F – Fsp) – (U – Usp)
Net protein utilization (%)	NPU = [I – (F -Fsp) – (U – Usp) / I] × 100
Biological value (%)	BV = [I – (F -Fsp) – (U – Usp) / I – (F -Fsp)] × 100

I: amount dietary protein ingested; F: protein excreted in the faeces of a subject other than the one on the protein-free diet; Fsp: protein excreted in the faeces of a subject on the protein-free diet; U: protein excreted in the urine of a subject other than the one on the protein-free diet; Usp: protein excreted in the urine of a subject on the protein-free diet; QAS: quantity of food served; QAR: quantity of food refused; DM: dry matter.

2.6. Statistical Analysis

Values are expressed as means ± standard deviation. Data were analyzed using Addinsoft software [16]. Figures were obtained using Windows 10 Excel. A Student's t-test for two independent samples was performed at the end of induction between the PCD control batch and the MID malnourished rat batch. A Duncan test and Pearson correlation were conducted at the conclusion of the nutritional rehabilitation phase to identify significant differences and establish existing relationships between nutritional parameters. The overall significance level was set at a P value of less than 0.05 for all tests. Principal component analysis and hierarchical ascending classification were employed to classify the various diets according to zootechnical and nutritional parameters.

3. RESULTS

3.1 Impact of PASLoc flours on zootechnical parameters

Table 4 displays the zootechnical parameters of the rats at the end of the malnutrition induction and nutritional rehabilitation phases. An analysis of the results indicates the efficacy of the moderate acute malnutrition induced in the rats through the consumption of the MID diet. Indeed, the Student's t test revealed that the rate of intake of the MID diet, as well as its food efficiency coefficient, were significantly lower ($P < 0.05$) than those of rats fed the PCD diet. Furthermore, the rats' consumption of the MID diet resulted in a significant weight loss, estimated at -22.36 ±6.47%. This weight loss had a negative impact on body growth (0.95 ±0.64 cm), abdominal circumference (-1.18 ±0.27 cm), and body mass index (0.33 ±0.03) compared with those on the PCD diet. In the nutritional rehabilitation phase, statistical analysis revealed significant differences ($P < 0.05$) with very high values in rats fed different PASLOC flours. Weight losses of -1.39 ±0.28 g/d were transformed into weight gains. In rats fed Pasloc2B and Pasloc1B, weight gains ranged from 3.46 ±0.65 g/d to 1.66 ±0.54 g/d, respectively, and were higher than in rats fed the PCD control diet. The feed efficiency coefficient, which was negative at the end of malnutrition induction, showed a significant difference with the PCD diet. FEC values were 23.62 ±2.83, 22.09 ±6.56, 33.59 ±5.61, 37.55 ±4.92, -11.86 ±6.37, and 9.61 ±10.81, respectively, for rats fed the Pasloc1A, Pasloc1B, Pasloc2A, Pasloc2B, MID, and PCD diets. As for the quantity of dry matter ingested, those ingested by rat batches Pasloc1A, Pasloc1B, Pasloc2A, and Pasloc2B, as well as the control diet, show a significant difference ($P < 0.05$), with significantly higher ingested quantities in the malnutrition induction phase. The quantities ingested were 8.94 ±0.77, 7.54 ±1.51, 7.06 ±0.31, 9.18 ±0.95, and 8.31 ±1.02, respectively, for Pasloc1A, Pasloc1B, Pasloc2A, Pasloc2B, and the control diet.

Table 4: Zootechnical parameters of rats at the end of malnutrition induction and nutritional rehabilitation stages

Parameters	Inducing malnutrition		Nutritional rehabilitation					
	MID	PCD	Pasloc 1A	Pasloc 1B	Pasloc 2A	Pasloc 2B	MID	PCD
TVP (%)	-22.36 ±6.47^{b}	15.93 ±7.65^{a}	25.26 ±2.94^{b}	20.84 ±5.03^{b}	28.28 ±1.96^{ab}	34.59 ±3.33^{a}	-9.27 ±5.82^{d}	8.08 ±8.86^{c}
WG (g/d)	-1.39 ±0.28^{b}	1.02 ±0.46^{a}	2.11 ±0.34^{b}	1.66 ±0.54^{bc}	2.39 ±0.49^{b}	3.46 ±0.65^{a}	-0.59 ±0.33^{d}	1.22 ±0.75^{c}
BL (cm)	0.95 ±0.64^{b}	2.35 ±1.35^{a}	0.61 ±0.62^{a}	0.35 ±0.21^{c}	0.33 ±0.17^{c}	0.55 ±0.33^{b}	0.15 ±0.30^{d}	0.65 ±0.30^{a}
BMI	0.33 ±0.03^{b}	0.40 ±0.03^{a}	0.41 ±0.03^{b}	0.40 ±0.04^{b}	0.40 ±0.06^{b}	0.49 ±0.05^{a}	0.32 ±0.02^{c}	0.41 ±0.03^{b}
AC (cm)	-1.18 ±0.27^{b}	1.25 ±0.58^{a}	2.53 ±1.00^{a}	2.20 ±1.10^{a}	3.35 ±2.14^{a}	3.30 ±0.90^{a}	1.38 ±0.85^{b}	2.00 ±0.87^{a}
DMI (g/d)	5.73 ±0.23^{b}	7.38 ±0.56^{a}	8.94 ±0.77^{ab}	7.54 ±1.51^{bc}	7.06 ±0.31^{c}	9.18 ±0.95^{a}	5.09 ±0.66^{d}	8.31 ±1.02^{abc}
FEC	-24.98 ±5.47^{b}	13.62 ±5.54^{a}	23.62 ±2.83^{b}	22.09 ±6.56^{b}	33.59 ±5.61^{a}	37.55 ±4.92^{a}	-11.86 ±6.37^{d}	9.61 ±10.81^{c}

On the same line, values with letters (a, b, c, and d) in superscript are significantly different at the 5% threshold.

3.2. Nutritional Potential of PASLoc Flours

Table 5 shows the nutritional potential of the PASLoc diets compared to the PCD diet. Duncan's test shows a significant difference (P < 0.05) in the nutritional potentials expressed by the different batches of rats fed the different PASLoc diets. PASLoc2B had the highest feed efficiency ratio (2.72 ±0.36), while PASLoc1B had the highest apparent digestibility (83.11 ±3.35%), true digestibility (94.76 ±1.04%), and net protein utilization (95.52 ±0.89%). Statistical analysis showed that PASLoc1B, PASLoc2A, and PASLoc2B diets had the same higher biological value (P < 0.05) than the PCD control diet.

Table 5. Nutritional potential of PASLoc diets

Régimes	PEC	Da (%)	Dr	BV (%)	PR (g)	NPU (%)
PASLoc1A	2,11 ±0,25^{b}	66,91 ±2,36^{c}	82,37 ±1,26^{c}	94,04 ±0,25^{c}	3,32 ±0,31^{c}	79,94 ±1,43^{d}
PASLoc1B	1,40 ±0,42^{c}	83,11 ±3,35^{a}	94,76 ±1,04^{a}	97,80 ±0,17^{a}	5,43 ±1,28^{b}	95,52 ±0,89^{a}
PASLoc2A	1,85 ±0,31^{bc}	70,55 ±0,99^{c}	80,98 ±0,64^{c}	97,83 ±0,03^{a}	5,01 ±0,20^{b}	81,66 ±0,61^{d}
PASLoc2B	2,72 ±0,36^{a}	76,96 ±3,42^{b}	89,02 ±1,63^{b}	97,88 ±0,15^{a}	4,85 ±0,80^{b}	89,80 ±1,52^{c}
PCD	0,48 ±0,54^{d}	85,56 ±2,90^{a}	93,24 ±1,36^{a}	95,89 ±0,24^{b}	7,91 ±1,61^{a}	92,21 ±1,56^{b}

On the same line, values with letters (a, b, c, and d) in superscript are significantly different at the 5% threshold.

Figure 1 depicts the principal component analysis (PCA) and hierarchical ascending classification (HAC) of the different PASLoc diets, as well as the zootechnical and nutritional parameters. It can be seen in Figure 1a that only PASLoc1B flour has a positive relationship with Axis 1 and 2 and all the parameters (FEC, WGA, CEP, VB, PNU, DR, DA, and RP), except for the amount of dry matter eaten. While the PASLoc 2A and 2B diets were only positively correlated with axis 2, the PCD control diet was positively correlated with axis 1. The PASLoc1A diet was not correlated with either axis 1 or 2. On the one hand, the hierarchical ascending classification (Figure 1B) shows a similarity between PASLoc1B flour and the PCD control diet, and on the other, a similarity between PASLoc 1A, 2A, and 2B.

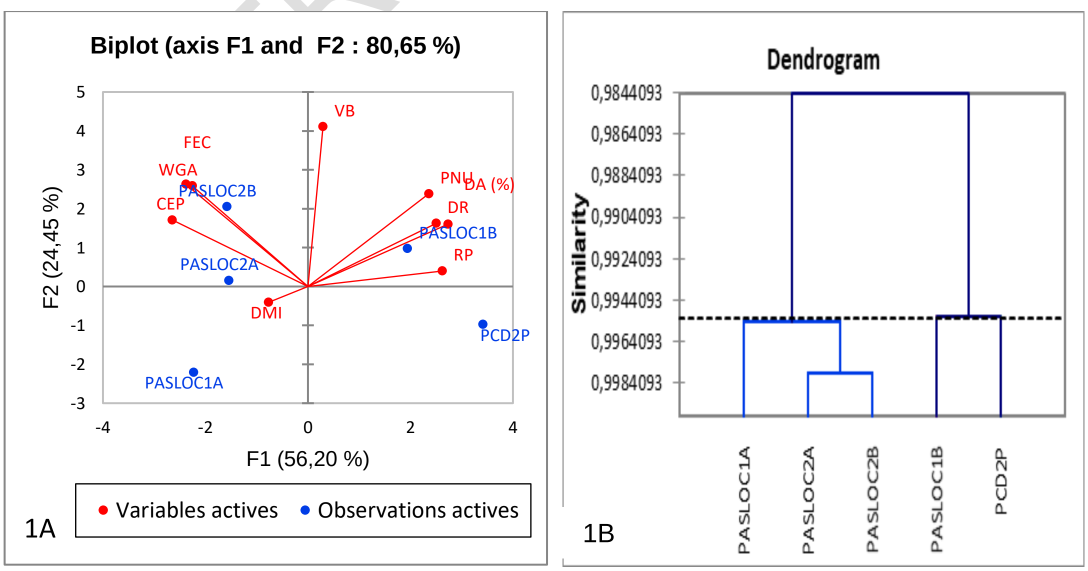

Figure 1: Classification of diets according to their zootechnical and nutritional potential; 1A: Factor analysis of the influence of zootechnical and nutritional parameters on the rehabilitation capacity of PASLoc diets; B: Grouping of different PASLoc diets according to their influence on zootechnical and nutritional parameters.

4. DISCUSSION

The aim of this study was to evaluate the ability of PASLoc diets consumed in nutritional rehabilitation centers in Côte d'Ivoire to reverse cases of moderate acute malnutrition. To assess this ability, an animal model using young Wistar rats was selected.

At the end of the induction period, all zootechnical parameters indicated that the rats in the MID batch had moderate acute malnutrition. The rate of induction of this malnutrition (-22.36 ±6.47%) was higher than the one that Borelli et al. [14] recommended, which is -20%. Cases of induction of malnutrition in rats consuming a similar 4% protein diet were reported by Ferrari et al. [17] and Eyzaguirre-Velásquez et al. [18] after 20 days of induction. The results showed that consumption of the different PASLOC diets resolved the malnutrition problems observed in the zootechnical parameters during the induction period. The variability in the nutritional makeup of the various PASLOC diets could account for this beneficial effect, which varied from diet to diet [12]. The PASLOC 2B diet's strong zootechnical potential in rats at the end of nutritional rehabilitation suggests that its consumption by children could be effective in controlling moderate acute malnutrition in children. Given that proteins are essential macronutrients for the body, these outcomes may be explicable by the high protein content of these various PASLoc diets. They contribute to metabolism and biological functions by building muscle, producing enzymes and hormones, and promoting growth [19]. The apparent and real digestibilities of the different PASLoc (1B, 2A, and 2B) and PCD diets clearly show that they are digestible by rats. In fact, according to Kamau et al. [20], actual digestibility is higher than apparent digestibility, which is an indicator of the nutritional quality of a diet. According to Wolter et al. [21], diets with less than 70% digestibility would not be able to meet energy requirements even with ad libitum distribution. PASLoc 1B flour had a higher true digestibility than the protein control diet and other PASLoc diets, making it more beneficial for malnourished children's energy management.

According to Hackler [22], the biological value of a food is a measure of the efficiency with which absorbed nitrogen is used. The biological values of PASLoc flours 1B, 2A, and 2B are higher than those of rats fed the protein control diet (PCD), implying that these flours, once consumed, will be used efficiently by the body for its metabolism. According to Bouafou [23], biological value is determined by the balance of essential amino acids. This balance can be attributed to the diversity of protein sources used in the formulation of the different compound flours.

The net protein utilization values of the different diets consumed by malnourished rats in the nutritional rehabilitation phase were lower than those determined for the diet, except for PASLoc 1B, which had a higher value. Nevertheless, the biological values and net protein utilization of all PASLoc meals exceeded the minimum recommended value (70%) for a good dietary protein mixture [24]. Consequently, consumption of these diets could have beneficial effects by activating cell growth and keeping the body in good health by meeting the body's protein requirements. Therefore, these foods are ideal for the nutritional rehabilitation of children suffering from protein-energy malnutrition.

Principal Component Analysis (PCA) and Hierarchical Ascending Classification (HAC) of the different PASLoc flours according to zoonotic and nutritional parameters allowed us to classify the PASLoc flours into two groups: the PASLoc 1A, 2A, and 2B group and the PASLoc 1B group. The fact that PASLoc 2A and 2B are positively correlated with most of the zoonotic and nutritional parameters, except for the amount of dry matter eaten, shows that these flours can help kids who are moderately to severely malnourished.

5. CONCLUSION

The aim of this study was to assess the ability of PASLoc flours, consumed in nutritional rehabilitation centers in Côte d'Ivoire, to restore cases of moderate acute malnutrition. The results of our study reveal that the various PASLoc diets have the ability to restore the dysfunctions observed in zootechnical parameters. In addition, the study of nutritional parameters reveals that PASLoc diets have a good capacity for digestion and assimilation. In-depth analysis of the results reveals that PASLoc 2A and 2B diets are better able to resolve moderate acute malnutrition problems. Looking ahead, it would be interesting to assess the impact of these different PASLoc diets on the biochemical, hematological, and histological constants of the rats.

ETHICAL APPROVAL

The authors of this study affirm that the research protocol adhered to the principles and guidelines for the care and use of laboratory animals in research as recommended by the U.S. National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23). The protocol was endorsed by the national ethics commission that granted approval for the research.

DEFINITIONS, ACRONYMS, ABBREVIATIONS

MID: Malnutrition inducing diet
PCD: Protein control diet
RWC: Rate of weight change
WG: Weight gain
BL: Body length
AC: Abdominal circumference
BMI: Body mass index
DMI: Dry matter intake
FEC: Feed efficiency coefficient
TPI: Total protein intake
Da: Apparent protein digestibility
Dr: Real protein digestibility
PEC: Protein efficiency coefficient
PR: Protein retention
NPU: Net protein utilization
BV: Biological value

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