



Dietary protein-energy restriction followed by restitution with or without fortification in growing rats

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Abstract

This study aimed to assess the effects of a fortified diet on nutritional indicators of growing rats undergoing a protein-energy restriction. For this purpose, three experiments of 30 days were carried out. The first experiment was performed to evaluate the effects of a protein-energy restriction on growth of 24 rats. The second experiment was intended to rehabilitate by restitution with appropriate diets 24 rats after a period of restriction. The last one was performed on 18 rats with a fortified restitution diet. After two weeks, protein-energy restriction induced a sharp drop in rat growth from the beginning to the end of the experiment. The average values of the nutritional characteristics of treated rats were lower than those of control rats. After protein-energy restitution, the daily average values of the nutritional characteristics of treated rats resume slightly. The restitution with fortification lead to a great resumption of growth and a consequent increase in nutritional characteristics of rats. In conclusion, for a protein-energy restitution to be effective, it must be fortified with a complex of essential amino acids and trace elements.

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Introduction

The food, due to its nutritional composition and nutritional effects, is an essential organic compound in the maintenance of human health and survival. Malnutrition, an abnormal physiological condition, is due to insufficient, unbalanced or excessive consumption of energy macronutrients (carbohydrates, proteins and lipids) and micronutrients essential for growth and physical and cognitive development (Amoikon *et al.*, 2016).

According to Subramanian *et al.* (2014), the possibilities of resuming growth after periods of malnutrition have long been explored. Since 1982, WHO has defined malnutrition as a pathological condition resulting from the deficiency or excess of one or more essential nutrients?

This condition can manifest clinically, or can be detectable by biochemical, anthropometric or physiological analyses. Thus, obesity and diabetes following over nutrition, according to N'diaye (2007), are specific cases of excess malnutrition.

On the other hand, acute malnutrition formerly known as protein-energy malnutrition (Beaufrère *et al.*, 1998), chronic malnutrition or stunting of statural growth, and overall malnutrition or underweight (Mwadianvita *et al.*, 2014) are cases of malnutrition by deficiency. There are also cases of malnutrition due to vitamin and essential mineral deficiency.

Numerous nutritional surveys have been conducted for decades on children to diagnose malnutrition (Amoikon *et al.*, 2016; Kouamé Konan *et al.*, 2017). In parallel to this work, laboratory experiments have been conducted on animal malnutrition to better understand the cellular mechanisms by which malnutrition occurs in children. However, it should be noted that rehabilitation, with feed fortification, has been subjected to a very few investigations in laboratory animals.

Therefore, the goal of this work is to investigate on the growth of malnourished rats, under fortified diet.

Materials and methods

Materials

Young male Wistar rats with an initial weight of between 55 and 60 g, were used in these three series of experiments. They came from the animal Laboratory of the UFR- Biosciences of the University of Félix Houphouët Boigny of Abidjan. Rats were contained in breeding cages arranged in a room, with a degree of hygrometry between 70 % and 80%, an average temperature of 25 ° C, with 12 hours of daylight and 12 hours of darkness. A Denver brand scale (Germany) was used to determine the weight of rats and feed.

Methods

Experimental models

After weaning, the young rats were subjected to a single feed, based on fish powder, in order to accustom them to the semi-synthetic experimental diet. Following this adaptation period, the rats were divided into 11 batches of 6 rats. In a series of three experiments, the control diet had 20% of fish proteins. The first experiment which lasted 15 days consisted of restriction diets containing 10% (P10), 5% (P5) and 0 % (P0) of proteins. R25 means that treated rats are fed with 25 % of control consumption, on a daily basis. Therefore, diets were labeled respectively R25P10, R25P5, R25P0 and a control diet (Control). The second experiment was composed of the same restriction diets (15 days) followed by a rehabilitation diet with the control feed (15 days). The third experiment (30 days) was composed of a restricted lot (R25P0) followed by a rehabilitation period, and a restricted lot (R25P0F) followed by a restitution fortified with 0.01 % of *Amin' total* in the drinking water. For all experiments, on day 30, animals fasted for 16 hours were weighed, anesthetized with ethyl urethane (20%) and sacrificed.

Formulation of diets

The control diet (Table 1), inspired from Garcin *et al.* (1984), and modified by Amoikon *et al.* (2010) was composed of herring fish meal and commercial cornstarch (Maizana). The set was completed by a

vitamin and mineral premix (Biacalcium, Laboratories Biové, France) and sunflower oil. All control diets were designed to meet the nutrient requirements of rats (Table 1). The preparation of animal feed consisted in mixing the various ingredients in a "Moulinex" brand mixer. These ingredients were then transferred to a saucepan, and after homogenization in one liter of water, the porridge obtained was subjected to cook on an electric stove brand "IKAMAG" (Germany), until it was setting in mass. The feed was placed on a plate and stored in a refrigerator (4 ° C). This preparation was renewed every two days. (Table 1) Five grams of each prepared feed were collected in duplicate and placed in an oven at 100 °C for 4 hours. After weighing, the average dry matter content was calculated. Every morning, between 7am and 8 am, the rats were fed and the water of the bottles was renewed. The feed allocated to each treatment is weighed and placed on the screen serving as a cover for the cages. The next day, the remaining feed was also weighed in order to determine the amount of feed ingested. The animals were weighed every two days. The different nutritional characteristics were obtained by calculation, according to table 2. For fortification, the malnourished rats receive, *per os*, from the 15th to the 30th day, *Amin' total* (Laprovét, France), at the dose of 0.01%. *Amin' total* is concentrate of essential amino-acid and oligo-

elements.

Statistical analyses of the results

The statistical data (average, standard deviation) are calculated from the Graph Pad Prism 5.1 "software". The comparison of the means obtained by the analysis of the variances (ANOVA) is followed by the Newman-Keuls multiple test (at the threshold of 5%). Two means are significantly different if the probability from the statistical tests is less than or equal to 0.05 ($P \leq 0.05$). The letters a, b, c, d, e, etc. in super script, follow the averages from the comparison tests in the tables. The averages followed by different letters on the same line are significantly different.

Results

Evolution of the growth of rats subjected to a protein-energy restriction diet

The first experiment consisted of four batches of rats, one of which was a control, fed on a 20% fish protein diet. The other three lots identified as R25P10, R25P5, R25P0 represent the R25P10 rats, rats receiving 25% of the consumption of controls with a 10% protein diet, rats R25P5, rats receiving 25% of the consumption of controls with a 5% protein diet and R25P0 rats, rats receiving 25% of the consumption of controls with a 0% protein diet (proteiprivate diet). Figure 1 represents growth curves during 15 days of protein-energy restriction.

Table 1. Composition of diets.

Criteria	Diets			
	Control	P10	P5	P0
Fish powder (g)	331.40	165.70	82.85	0.00
Corn flour (g)	342.60	508.30	591.15	674.00
Sugar (g)	275.00	275.00	275.00	275.00
Premix (g)	1.00	1.00	1.00	1.00
Sunfloweroil (mL)	50.00	50.00	50.00	50.00
Total (g)	1000.00	1000.00	1000.0	1000.0
Digestible energy (Kcal)	4246.0	4246.0	4246.0	4246.0

Control diets: 20% proteindiet; P10: 10% proteindiet; P5: 5% proteindiet; P0: 0% proteindiet (proteiprivate); Energy level of the diets: 4246 kcal / kg DM. Premix: Biacalcium, Laboratories Biové, France.

These curves represent the evolution of the growth of rats in different groups depending on the duration of the experiment. This figure shows that the growth of control rats is higher than that of rats on R25P10, R25P5 and R25P0 diets. The average weight of all

batches of rats subjected to protein-energy restriction decreases from the beginning to the end of the experiment. The lowest growth was seen in R25P0 rats. This growth of rats is proportional to the applied protein rate (Fig. 1).

Table 2. Expression of nutritional characteristics of rats.

Nutritional characteristics	Mathematical Expressions
Feed intake (FI) (g)	Feed given – Feed refused
Material moisture content (MMC) %	$[(\text{Fresh Material} - \text{Dry Matter}) / \text{Fresh Material}] \times 100$
Dry matter ratio (DM) %	100 – MMC
Dry matter intake (DMI/) (g)	FI x DM
Protein intake (PI) (g)	DMI x % protein of diet
Average weight gain (AWG) (g)	Final weight – Initial weight
Feed efficiency (FE)	AWG / DMI
Protein efficiency (PE)	AWG / PI

Nutritional characteristics of rats at the end of protein-energy restriction

The average nutritional characteristics of treated rats (R25P10, R25P5 and R25P0) are generally lower than those of control animals (Table 3). The final weight (FW) of R25P10 rats (55.45 ± 1.80 g), R25P5 (52.75 - 6.3 g) and R25P0 (51.41 ± 1.70 g) is lower than that of

control rats (89.70 ± 7.30 g). The value of the dry matter ingested (DMI) of R25P10 rats (1.52 ± 0.00 g), R25P5 (1.41 ± 0.00 g) and R25P0 (1.70 ± 0.51 g) is lower than that of control rats (5.79 ± 0.00 g). The body weight gain (BWG) of R25P10 rats (-1.19 ± 0.05 g), R25P5 (-0.85 ± 0.04 g) and R25P0 (-1.51 ± 0.17 g) is lower than that of control rats (2.18 ± 0.47 g).

Table 3. Average value of nutritional characteristics of control rats and experimental rats.

Criteria	Diets composition				
	Control (n = 6)	R25P10 (n = 6)	R25P5 (n = 6)	R25P0 (n = 6)	P Value
IW (g)	58.63±1.8 ^a	58.80±1.7 ^a	60.38±7.0 ^a	60.66±3.55 ^a	0.59
FW (g)	89.70±7.3 ^a	55.45±1.8 ^b	52.75±6.3 ^b	51.41±16 ^b	0.01
DMI (g)	5.79±0.00 ^a	1.52±0.00 ^b	1.41±0.00 ^b	1.51± 0.00 ^b	0.00
BWG (g)	2.18±0.47 ^a	-1.19±0.05 ^b	-0.85±0.04 ^b	-1.51±0.17 ^b	0.00
FE	0.38±0.08 ^a	-0.78±0.04 ^b	-0.61± 0.03 ^b	-1.00± 0.11 ^b	0.00
ITP	1.16±0.00 ^a	0.15±0.00 ^b	0.7±0.00 ^c	-	0.00
PE	1.88±0.41 ^a	-7.83±0.36 ^b	-12.17± 0.61 ^c	-	0.00
DE (kcal)	24.59±0.00 ^a	6.44±0.00 ^b	5.97± 0.00 ^b	6.42±0.00 ^b	0.00

Duration of experience: 15 days; ANOVA followed by the Multiple Comparison Test of Newman-Keuls averages at the 5% threshold. averages are followed by letters in super script (a, b, c, d, etc.); averages with different letters are statistically different; P - 0.05: significant difference between averages; Control rats (20 % fish proteins); R25P10: batch of rats receiving 25% of the consumption of control rats with 10% fish protein diet; R25P5: batch of rats receiving 25% of the consumption of control rats; and 5% fish protein diet; R25P0: batch of rats receiving 25% of the consumption of control rats with the 0% fish protein diet; IW: initial weight; FW: final weight; DMI: dry material ingested; BWG: body weight gain; FE: feed efficiency; ITP: ingested total protein; PE: protein efficiency; DE: digestible energy. n : number of rats.

The feed efficiency (FE) value of the rats tested R25P0 (-1.00 ± 0.11), R25P5 (-0.61 ± 0.03) and R25P10 (-0.78 ± 0.04) is lower than those of control rats (0.38 ± 0.08). The value of the ingested total protein (ITP) of control rats (1.16 ± 0.00 g) is higher than that of R25P10 rats (0.15 ± 0.00 g), R25P5 (0.7

± 0.001 g) and R25P0. Protein efficiency (PE) value changes in the same order as those of ITP.

The value of the protein efficiency (PE) of control rats (1.88 ± 0.41) is higher than those of R25P10 (-7.83 ± 0.36) and R25P5 (-12.17 ± 0.61).

Table 4. Average value of growth characteristics of control rats and experimental rats.

Criteria	Diets composition				P
	Control	R25P10	R25P5	R25P0	
	(n = 6)	(n = 6)	(n = 6)	(n = 6)	
IW 1 (g)	56.29±2.5 ^a	52.60±5.4 ^a	51.25±0.48 ^a	52.90±3.1 ^a	0.72
IW 2 (g)	82.42±3.6 ^a	43.38±2.8 ^b	40.00±1.7 ^b	35.16±1.6 ^b	0.00
FW (g)	83.29±7.6 ^a	43.16±3.8 ^b	39.81±2.8 ^b	34.85±1.6 ^b	0.00
DMI (g)	4.84±0.00 ^a	2.68±0.00 ^c	2.69±0.00 ^c	3.12±0.00 ^b	0.00
BWG (g)	0.87±0.21 ^a	-0.22±0.20 ^b	-0.19±0.09 ^b	-0.31±0.12 ^b	0.00
FE	0.18±0.04 ^a	-0.08±0.07 ^b	-0.07±0.03 ^b	-0.10±0.04 ^b	0.00
ITP (g)	0.97±0.00 ^a	0.54±0.00 ^c	0.54±0.00 ^c	0.62±0.00 ^b	0.00
PE	0.90±0.22 ^a	-0.41±0.13 ^b	-0.35±0.19 ^c	-0.50±0.21 ^b	0.03
DE (kcal)	20.63±0.00 ^a	11.42±0.00 ^c	11.46±0.00 ^c	13.30±0.00 ^b	0.00

Duration of experiment: 30 days; ANOVA followed by the Multiple Comparison Test of Newman-Keuls averages at the 5% threshold. averages are followed by letters in super script (a, b, c, d, etc.); averages with different letters are statistically different; P - 0.05: significant difference between averages; Control rats (20 % fish proteins); R25P10: batch of rats receiving 25% of the consumption of control rats with 10% fish protein diet; R25P5: batch of rats receiving 25% of the consumption of control rats with 5% fish protein diet; R25P0: batch of rats receiving 25% of the consumption of control rats with the 0% fish protein diet; IW: initial weight; FW: final weight; DMI: dry material ingested; BWG: body weight gain; FE: feed efficiency; ITP: ingested total protein; PE: protein efficiency; DE: digestible energy. n: number of rats.

The value of digestible energy (DE) of rats subjected to R25P10 diets (6.44 ± 0.00 Kcal), R25P5 (5.97 ± 0.00 Kcal) and R25P0 (6.42 ± 0.00 Kcal) is lower than that of control rats (24.59 ± 0.00 Kcal). After two weeks of protein-energy restriction, the average

daily values of nutritional characteristics (PF, GPC, MSI, EBI, PTI, CEA and CEP) of treated rats are lower than those of control rats (P=0.001). Rat growth declines over time for rats subjected to protein-energy restriction diets, regardless of protein levels.

Table 5. Average value of nutritional characteristics of rats under protein-energy restriction and subjected to protein-energy restitution with or without fortification.

Criteria	Diets composition			P
	Control	R25P0	R25PoF	
	(n = 6)	(n = 6)	(n = 6)	
IW (g)	56.29±3.17 ^a	52.90±3.17 ^a	49.13±3.02 ^a	0.28
FW (g)	82.57±7.6 ^a	35±1.6 ^b	105.50±12 ^a	0.00
IDM (g)	4.84±0.00 ^a	3.12±0.00 ^b	6±0.00 ^a	0.00
BWG (g)	0.87±0.21 ^b	-0.31±0.12 ^c	3.39±0.45 ^a	0.00
FE	0.18±0.04 ^a	-0.10±0.03 ^c	0.56±0.07 ^b	0.00
ITP (g)	0.99±0.00 ^a	-	1.20±0.00 ^b	0.00
PE	0.90±0.22 ^a	-	2.6±0.38 ^a	0.00
DE (Kcal)	20.55±0.00 ^b	13.25±0.00 ^c	25.53±0.00 ^a	0.00

Duration of experience: 30 days; ANOVA followed by the Multiple Comparison Test of Newman-Keuls averages at the 5% threshold. averages are followed by letters in super script (a, b, c, d, etc.); averages with different letters are statistically different; P - 0.05: significant difference between averages; Control rats (20% protein); R25P0: batch of rats receiving 25% of the 0% protein diet and subjected to protein-energy restitution; R25PoF: batch of rats receiving 25% of the diet consumption at 0% protein and subjected to protein-energy restitution with fortification; IW: initial weight; FW: final weight; DMI: dry material ingested; BWG: body weight gain; FE: feed efficiency; ITP: ingested total protein; PE: protein efficiency; DE: digestible energy; n = 6.

Evolution of the growth of control rats and protein-energy restitution rats

The second experiment consists of four batches of rats, one of which is a control, fed on a 20% fish protein diet. The other three lots identified as R25P10, R25P5, R25P0 represent the R25P10 rats, rats receiving 25% of the consumption of controls with a 10% protein diet, rats R25P5, rats receiving 25% of the consumption of controls with a 5% protein diet and R25P0 rats, rats receiving 25% of the consumption of controls with a 0% protein diet

(proteoprive diet). After 15 days of protein-energy restriction, the three lots receive, *ad libitum*, diets of 10%, 5% and 0% of fish protein, respectively, for two weeks. Figure 2 shows the growth curves at the end of the protein-energy restitution period of rats R25P10, R25P5, R25P0 and Control.

The growth of R25P10, R25P5 and R25P0 rats resumes very timidly. Protein-energy restitution has not been able to correct the drop in growth caused by protein-energy restriction (Fig.2).

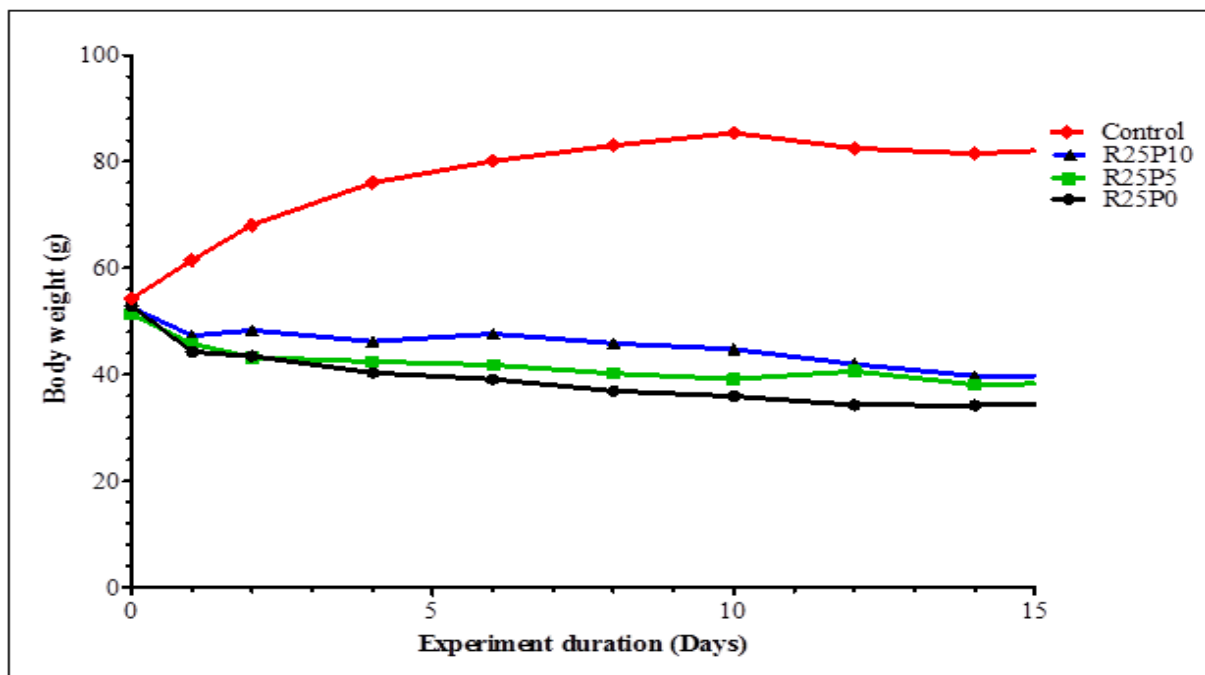


Fig. 1. Evolution of body weight of control rats and protein-energy restriction rats.

Duration of experience: 15 days of protein restriction; ANOVA followed by the Multiple Comparison Test of Newman-Keuls averages at the 5% threshold. averages are followed by letters in super script (a, b, c, d, etc.); averages with different letters are statistically different; $P \leq 0.05$: significant difference between averages; Control rats (20% fish protein); R25P10: batch of rats receiving 25% of the diet consumption at 10% protein; R25P5: batch of rats receiving 25% of the diet consumption at 5% protein; R25P0: batch of rats receiving 25% of the diet at 0% protein diet (proteoprive diet); $n=6$.

Nutritional characteristics of control rats and experimental rats at the end of protein-energy restitution

The nutritional characteristics of the treated rats (R25P10, R25P5 and R25P0) are lower than those of controls (Table 4). Despite the protein-energy restitution, all nutritional characteristics of rats are lower than those of control ones. The final weight (FW) of the R25P10 rats (43.16 ± 3.8 g), R25P5 (39.81

± 2.8 g) and R25P0 (34.85 ± 1.6 g) is statistically lower than those of control rats (83.29 ± 7.60 g). The value of dry matter ingested (DMI) of rats subjected to the R25P10 return regimes (2.68 ± 0.00 g), R25P5 (2.69 g), R25P0 (3.12 ± 0.00 g) is lower than that of control rats (4.84 ± 0.00 g). The average body weight gain (BWG) of R25P10 rats (-0.31 ± 0.12 g), R25P5 (-0.09 ± 0.09 g) and R25P0 (-0.22 ± 0.02 g) is lower than that of control rats (0.87 ± 0.21 g). The value of

the Feed efficiency (FE) of rats subjected to the R25P10 (-0.08 ± 0.04), R25P5 (-0.07 ± 0.03) and R25P0 (-0.10 ± 0.07) is lower than that of control rats (0.18 ± 0.04). The value of ingested total proteins

(ITP) of rats subjected to the R25P10 restitution regimens (0.54 ± 0.001 g), R25P5 (-0.54 ± 0.00 g) and R25P0 (0.62 ± 0.00 g) is lower than that of control rats (0.97 ± 0.00 g).

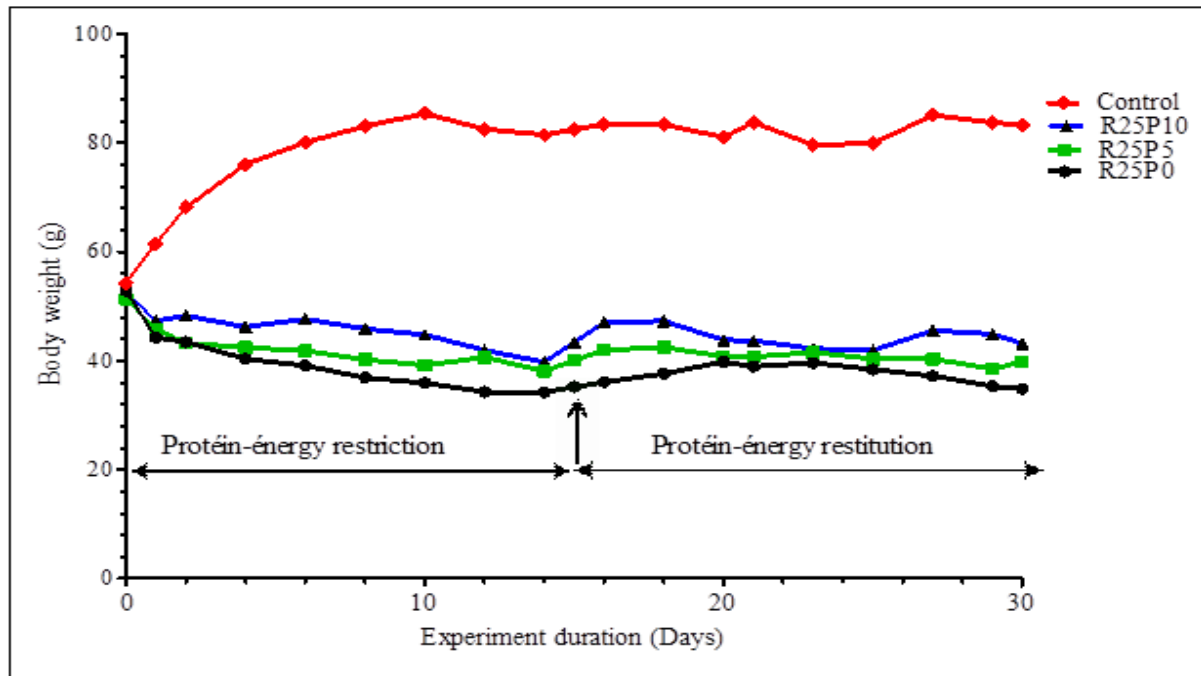


Fig. 2. Evolution of body weight of control rats and experimental rats under restriction and restitution.

Duration of the experiment: 30 days; ANOVA followed by the Multiple Comparison Test of Newman-Keuls averages at the 5% threshold. Averages are followed by letters in super script (a, b, c, d, etc.); averages with different letters are statistically different; $P \leq 0.05$: significant difference between averages; Control rats (20 % proteins); R25P10: batch of rats receiving 25% of the diet's consumption at 10% protein. R25P5: batch of rats receiving 25% of the diet's consumption at 5% protein; R25P0: batch of rats receiving 25% of the diet's consumption at 0% protein; the arrow indicates the end of the restriction period and the beginning of the period of food protein-energy restitution; $n=6$.

The value of protein efficiency (PE) of rats subjected to R25P10 (-0.41 ± 0.13 g), R25P5 (-0.35 ± 0.19 g) and R25P0 (-0.50 ± 0.21 g) is lower than that of control rats (0.90 ± 0.22). The average value of digestible energy (DE) of control rats (20.63 ± 0.00 Kcal) is higher than those of rats subjected to R25P10, (11.42 ± 0.00 Kcal), R25P5 (11.46 ± 0.00 Kcal) and R25P0 ($13.30 - 0.001$ Kcal) (Table 4). After a period of 15 days of protein-energy restriction and two weeks of protein-energy restitution, the average values of nutritional characteristics (FW, BWG, DMI, IPT, FE, PE, DE) of control rats are higher than those of rats subjected to protein-energy restitution ($p \leq 0.05$). The growth of rats picks up very slowly during protein-energy restitution.

Evolution of the growth of rats under a protein-energy restriction and rats subjected to protein-energy restitution with or without fortification

This 3rd experiment consists of a group of control rats (Control: 20% fish protein), and a group of rats subjected to protein-energy restriction (R25P0). The latter group, after 15 days of experiment, is divided into two subgroups (R25P0 and R25PoF). The 1st subgroup is rats that undergo a simple restitution, with the control diet (R25P0). The second subgroup is subjected to a fortified restitution (R25PoF) with the "Amin' total" concentrate. Figure 3 shows growth curves during the two-week restriction, and during the proteino-energy restitution period with or without fortification. During this period, the growth of rats

subjected to fortified restitution is faster than that of rats subjected to restitution (without fortification), and that of Control one. Ten days after restitution, the curve of rats subjected to protein-energy restitution with fortification passes above that of control rats. The growth of rats subjected to the fortified diet grow

so rapidly that it exceeds that of control rats, whose growth remains almost stationary. The fortification of diets during feed restitution allows a strong resumption of growth after the period of protein-energy restriction (Fig.3).

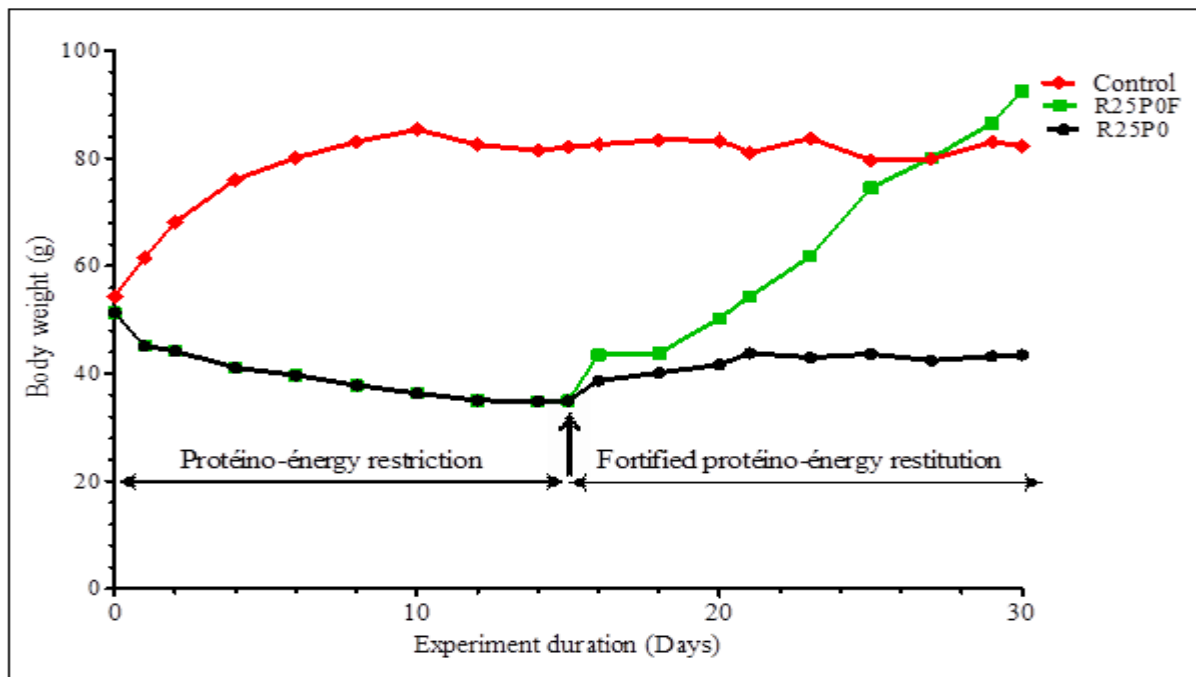


Fig. 3. Evolution of body weight of rats under protein-energy restriction and subjected to protein-energy restitution with or without fortification.

Duration of the experiment: 30 days; ANOVA followed by the Multiple Comparison Test of Newman-Keuls averages at the 5% threshold. averages are followed by letters in super script (a, b, c, d, etc.); averages with different letters are statistically different; $P < 0.05$: significant difference between averages; Control rats (20% protein); R25Po: batch of rats receiving 25% of the 0% protein diet subjected to restitution; R25PoF: batch of rats receiving 25% of the 0% protein diet and subjected to protein-energy restitution with fortification; The arrow indicates the end of the restriction.

Nutritional characteristics of rat's underprotein-energy restriction and rats subjected to protein-energy restitution with or without fortification

The difference between the nutritional indicator values of control rats (20 % fish protein) and those of rats under protein-energy restriction with an R25Po diet are significant ($P \leq 0.0001$). The nutritional indicators values of rats subjected to fortified protein-energy restitution with a R25PoF diet are higher than those of control rats (Table 5). Fortified protein-energy restitution provoked an increase of values of nutritional characteristics. The final weight (FW) of control rats (82.57 ± 7.6 g) is not statistically different

from that of rats subjected to protein-energy restitution with fortification R25PoF (105.00 ± 12 g), but higher than that of rats subjected to protein-energy restitution without fortification ($P \leq 0.0001$). The value of ingested dry matter (DMI) of control rats (4.84 ± 0.00 g) is greater than that of R25Po rats (3.12 ± 0.00 g) and smaller than that of R25PoF rats (6.00 ± 0.00 g) ($P \leq 0.001$). The average body weight gain (BWG) of R25PoF rats (3.39 ± 0.45 g) is higher than that of control rats (0.87 ± 0.21 g) and R25Po rats (-0.31 ± 0.12 g) ($P \leq 0.0001$). The value of the feed efficiency coefficient (FE) of control rats (0.18 ± 0.04) is higher than that of R25Po rats ($-0.10 \pm$

0.038) and lower than that of R25Po rats (0.25 ± 0.74) ($P \leq 0.0001$). The value of total ingested proteins (ITP) of control rats (0.99 ± 0.0001 g) is lower than that of R25PoF rats (1.20 ± 0.00 g) ($P \leq 0.0001$). The value of protein efficacy (PE) of control rats (0.90 ± 0.22) is lower than that of R25PoF rats (2.6 ± 0.38). The average value of the digestible energy (DE) of control rats (20.55 ± 0.00 Kcal) is lower than that of R25PoF rats (25.53 ± 0.00 Kcal) and higher than that of R25Po rats (13.25 ± 0.00 Kcal) (Table 5).

Discussion

The protein-energy restriction induces a sharp drop in rat growth from the beginning to the end of the experiment, although control rats have normal growth. After two weeks of protein-energy restriction, the average values of the nutritional characteristics of treated rats are lower than those of control rats. These results are corroborated by those of Kirsch *et al.* (1968) who reported that rats fed 5% mixed protein develop clinical, biochemical and histological disorders similar to those observed in children with kwashiorkor. The growth of these animals is significantly altered, with edema on the body. Even Prost *et al.* (1979) worked on 300 male Wistar rats, weighing 100 g at the beginning of the experiment, and divided into two batches. A control lot with 20% casein, for 70 days, and the experimental lot with a 2% casein diet for 34 days, then for 36 days with the control lot diet. The results of this experiment show that from the beginning of malnutrition, there is a shutdown of growth in experimental rats. This corroborates the results presented in this work.

Many works confirm the results presented in this work. Durand et Bourgeaux (1976) conducted an experiment in which 70 g male rats were restricted, either protein or energy intake of the ration, compared to a control batch receiving a balanced diet containing 16% protein. Protein restriction promotes fresh weight and adipose tissue, while energy restriction promotes the weight of the musculature and viscera. Rerat and Desmoulin (1970) working on

70 Wistar rats, weighing 66.5 g, and divided into 7 batches, each of which receives a different level of energy, nitrogen or energy alone. In addition to a control lot receiving a well-balanced diet of 13% nitrogen material (fish meal fortified with DL-Methionine). Under these conditions, the authors noted a decrease in the rate of growth and daily retention of energy and nitrogen in each daily intake of a well-balanced restriction diet.

During protein-energy restitution, rat growth resumes very slowly. After two weeks of protein-energy restriction, the average daily values of the nutritional characteristics of treated rats resume slowly, but remain lower than those of control rats. The results presented in this work are in agreement with those of Prost *et al.* (1979) which showed that the balanced re-feeding of rats allows for a greater recovery in growth, compared to controls. However, at the end of the experiment, the weight of experimental animals is one third less than that of control animals. Lake and Lake Scanzi (1984) conducted a study on two batches of rats weaned at 21 days. The malnourished lot receives a standard 26-day diet (15% casein). This malnourished lot is compared to a control lot that receives the standard diet, *ad libitum*. While body weight growth is linear in controls, it is zero in malnourished people, and resumes with the standard diet (after restitution). These results are consistent with those presented in this experiment. Similarly, Rerat and Desmoulin (1971) working on 70 Wistar rats, weighing 66.5 g, and divided into 7 batches, each of which receives a different level of energy and nitrogen or energy alone. In addition to a control lot receiving a well-balanced diet of 13% nitrogen material (fish meal fortified with DL-Methionine). Nitrogen deficiency only makes up for a small portion of the growth and nitrogen deposition delay in experimental animals.

Work on experimental malnutrition has yielded the same results on other animal species. Lister and McCance (1967) showed that severely under-nutrient pigs, after weaning, fail to reach their full stature, even after a long period of rehabilitation. These same

authors conclude that most of the disturbances produced by caloric deficiency, such as calcified tissues, are restored after the rehabilitation of the animals with a balanced diet. But other organs, especially teeth, are irreversibly and permanently damaged. The fortification of diets during food restitution has allowed a strong recovery in growth, reaching the same level as that of Control one. Fortified protein-energy restitution also results in an increase in the values of the nutritional characteristics of treated rats.

Conclusion

After two weeks, protein-energy restriction induces a sharp drop in rat growth from the beginning to the end of the experiment. The average values of the nutritional characteristics of treated rats are lower than those of control rats. After two weeks of protein-energy restitution, the average daily values of the nutritional characteristics of treated rats resume slowly, but remain lower than those of control rats. The protein-energy restriction was so deep on the animals (growth) that 15 days of restitution experience do not seem long enough for full rehabilitation. The return with fortification leads to a great resumption of growth and a consequent increase in nutritional characteristics of rats.

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