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RESEARCH ARTICLE

EFFICIENCY OF BALANCED AND FORTIFIED DIET ON GROWTH AND VALUES OF SERUM PARAMETERS IN YOUNG RAT PROTEIN DEFICIENCY

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ABSTRACT

This work consists of evaluating the effects of a balanced and fortified diet on the nutritional characteristics and values of serum metabolites in protein-deficient rats. A series of three experiments, with 11 lots of 6 rats is conducted, with a control diet containing 20% of protein. The first experiment which lasts 15 days consists of restriction diets containing 10% (P 10), 5% (P 5) and 0% (P 0) of proteins, relative to the control. The second experiment is composed of the same restriction diets (15 days) followed by a rehabilitation diet with the control feed (15 days). The third experiment (30 days) consisted of a restricted period (P0) followed by a single restitution, and a restricted lot (P0F) followed by a restitution fortified with 0.01% of Amin' total in the drinking water. The results obtained indicate that the body weight gain of malnourished rats P10 (1.11 ± 0.34 g), P5 (0.53 ± 0.45 g), P0 (-1.01 ± 0.28 g) is lower than that of the control rats (2.69 ± 0.49 g). The rehabilitation experiment has a minimal impact on body weight gain. On the other hand, fortified rehabilitation rats (P0F) had a body weight gain (3.17 ± 0.85 g) greater than those of rehabilitated rats (P0) (0.57 ± 0.25 g) and control rats (1.36 ± 0.49 g). Malnutrition has variable effects on the average values of blood parameters. In conclusion, protein malnutrition reduces growth in rats. Rehabilitation with a balanced diet alone is not enough to restore nutritional disturbances. To achieve a fast growth, it is necessary to associate with the balanced diet, a fortification with a food supplement enriched with nutrients (essential amino acids, vitamins, trace elements).

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INTRODUCTION

Malnutrition is due to insufficient, excessive or unbalanced consumption of macronutrients (carbohydrates, proteins and fats) and micronutrients essential for growth, physical and cognitive development. Many nutritional surveys have been conducted on children to diagnose malnutrition (Amoikon *et al*., 2016, Kouamé *et al*., 2017). According to Subramanian *et al.* (2014), the possibilities of resuming growth after periods of malnutrition have long been studied. Many experiments have focused on protein, energy and protein-energy malnutrition in rats (Bertrand 1991, Kilicalp *et al.* 2005). Prost *et al.* (1979) reported that a balanced dietary treatment could restore enzymatic activity of lipases and phospholipases in rats. Durand and Bourgeaux (1976) showed in rats undernourished for two years that growth rehabilitation and cellular multiplication can take place.

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The aim of this work is to explore the consequences of experimental malnutrition in young growing rats and to overcome them by rehabilitation with a balanced and fortified diet.

MATERIAL AND METHODS

Material: Rats (*Rattus norvegicus*, Muridae, L. 1753) in growth, with an initial weight of between 55 and 60 g, of male sex, are used in this series of experiments. They come from the pet shop of the UFR Biosciences of the Félix Houphouët-Boigny University of Cocody-Abidjan. The animals are contained in breeding cages arranged in a room, with a degree of hygrometry of 70% to 80%, a temperature of 25 ° C, with 12 hours of daylight and 12 hours of darkness. A Denver brand scale (Germany) is used to determine the weight of rats and feed. The biological criteria are assayed by a multiparametric analyzer (HITACHI 902-Roche, Japan).

Methods

Experimental models: After weaning, the young rats are subjected to a single food, based on fish powder, in order to

accustom them to the semi-synthetic experimental diet. Following this adaptation period, the rats are divided into 11 batches of 6 rats. In a series of three experiments, the control diet has 20% of protein. The first experiment which lasts 15 days consists of restriction diets containing 10% (P 10), 5% (P 5) and 0% (P 0) of proteins, relative to the control. The second experiment is composed of the same restriction diets (15 days) followed by a rehabilitation diet with the control food (15 days). The third experiment (30 days) is composed of a restricted lot (P0) followed by a rehabilitation period, and a restricted lot (P0F) followed by a restitution fortified with 0.01% of tl *Amin' total* in the drinking water. On days 15 and 30, animals fasted for 16 hours are anesthetized with ethyl urethane (20%) and sacrificed; and the collected blood is frozen at -20 ° C for analysis. After laparotomy, the rat organs such as the heart, both kidneys, liver, spleen and abdominal fat are carefully removed, rinsed with NaCl (9 ‰), weighed and preserved in formalin. The different dosages of serum metabolites are carried out at the Biochemistry Laboratory of the University Hospital of Treichville in Abidjan.

Formulation of diets

The control diet (Table 1), inspired from Garcin *et al.* (1984), and modified by Amoikon *et al.* (2010) is composed of fish meal (herring) and commercial cornstarch (Maizana). The set is completed by a vitamin and mineral premix (Biacalcium, Laboratoires Biové, France) and sunflower oil. All diets are designed to meet the nutrient requirements of rats. The preparation of animal feed (Table 1) consists in mixing the various ingredients in a "Moulinex" brand mixer. These ingredients are then transferred to a saucepan, and after homogenization in one liter of water, the slurry obtained on cooking is subjected to an electric stove brand "IKAMAG" (Germany), until it's setting in mass. The food is placed on a plate and stored in a refrigerator (4 ° C). This preparation is renewed every two days.

Table 1. Composition of diets

Ingrédients	Diet treatments			
	Control	P10	P5	P0
Fish meal (g)	331.40	165.70	82.85	0.00
Cornstarch (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
Sunflower oil (mL)	50.00	50.00	50.00	50.00
Total (g)	1000.00	1000.00	1000.00	1000.00
Gross energy (Kcal)	4246.00	4246.00	4246.00	4246.00

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

Five grams of each prepared food are collected in duplicate and placed in an oven at 100 °C for 4 hours. After weighing, the average dry matter content is calculated. Every morning, between 7am and 8am, the rats are fed and the water of the bottles is renewed. The food allocated to each treatment is weighed and placed on the screen serving as a cover for the cages.

The next day, the food remains are also weighed to determine the amount of food ingested. The animals are weighed once every two days. The different nutritional characteristics are obtained by calculation, according to table 2. For fortification, the malnourished rats receive, *per os,* from the 15th to the 30th day, *Amin' total* (Laprovet, France), at the dose of 0.01%.

Table 2. Expression of nutritional characteristics

Nutritional characteristics	Mathematical Expressions
Feed intake (FI) (g)	Feed given – Feed refused
Material moisture content (MMC) %	[(Fresh Material - Dry Matter) / Fresh Material] x 100
Dry matter ratio (DM) %	100 – MMC
Dry matter intake (DMI) (g)	(FI x DM) / 15 days / 6 rats
Protein intake (PI) (g)	DMI x % proteins in diet
Average weight gain (AWG) (g)	(Final weight – Initial weight) / 15 days / 6 rats
Feed efficiency (FE)	AWG / DMI
Protein efficiency (PE)	AWG / PI

Expression and statistical analysis of the results

The statistical data (average, standard deviations) are calculated from the GraphPad Prism 5.1 "software". The comparison of the means obtained by the analysis of the variances (ANOVA) is followed by the Newman-Keuls 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

Effects of protein restriction on the nutritional characteristics of rats: Table 3 indicates that the differences between the mean values of the nutritional indicators of the rats subjected to the diets corresponding to the protein levels studied (10%, 5% and 0% of proteins) and those of the control rats are significant ($p<0.05$) at the end of the protein restriction period. The final weights (PF) of the protein restricted rats, P10 (74.06±3.30 g), P5 (65.35±5.70 g) and P0 (41.35±2.70 g) are weaker than that of the control rats (96.35±8.50 g). Mean ingested dry matter (IDM) values for P10 rats (5.93±0.00 g), P5 (5.06±0.00 g) and P0 (3.00±0.00 g) are inferior to that of control rats (6.42±0.00 g). The body weight gains (BWG) of batches P10 (1.11±0.34 g), P5 (0.53±0.45 g) and P0 (-1.01±0.28 g) are lower than that of control rats (2.69±0.49 g). The feed efficiency coefficients (FE) of the P10 (0.19±0.09), P5 (0.10±0.09) and P0 (-0.34±0.09) rats are also lower than that of the control rats (0.42±0.07). Total Ingested Protein (TIP) values for P10 (1.19±0.00 g) and P5 (1.01±0.00 g) are lower than controls one (1.28±0.00 g). Protein Efficiency (PE) values of P10 (0.94±0.57) and P5 (0.52±0.08) rats are lower than that of control rats (2.09±0.38). Ingested gross energy (IGE) values decreases from control rats (27.26±0.00 Kcal) to rats fed the proteiprive diet (12.74±0.00 Kcal), via P10 rats (25.18±0.00 Kcal) and P5 rats (21.48±0.00 Kcal), with significant differences. Protein restriction causes the values of the nutritional characteristics of the rats to drop.

Effects of protein restriction on mean values of serum metabolites in rats : At the end of the protein restriction period, the values of some serum metabolites do not vary significantly ($P > 0.05$) compared to controls ones. This is the case of uric acid, glucose, creatinine, total cholesterol, HDL cholesterol and triglycerides (Table 4). In contrast, the mean values of urea in malnourished rats P10 (0.25±0.02 g/L), P5 (0.18±0.04 g/L) and P0 (0.16±0.02 g/L) are lower than control rats (0.26±0.05 g/L) ($P = 0.05$). Mean total bilirubin values of animals in lots P10 (13.00±5.50 mg/L), P5 (7.80±0.73 mg/L)

Table 3. Average value of nutritional characteristics of rats at the end of protein restriction

Criteria	Diet treatments				
	T (6)	P10 (6)	P5 (6)	P0 (6)	P Value
IW (g)	57.00±5,72[a]	57.41±6.69[a]	57.40±5.36[a]	56.50±4.85[a]	0.99
FW (g)	96.35±8.50[a]	74.06±3.30[b]	65.35±5.70[b]	41.35±2.70[c]	0.00
DMI (g)	6.42±0.00[a]	5.93±0.00[b]	5.06±0.00[c]	3.00±0.00[d]	0.00
AWG (g)	2.69±0.49[a]	1.11±0.34[b]	0.53±0.45[c]	-1.01±0.28[d]	0.00
FE	0.42±0.07[a]	0.19±0.09[b]	0.10±0.09[c]	-0.34±0.09[d]	0.00
TPI (g)	1.28±0.00[a]	1.19±0.00[b]	1.01±0.00[c]	nd	0.00
PE	2.09±0.38[a]	0.94±0.57[b]	0.52±0.08[c]	nd	0.00
GEI (kcal)	27.26±0.00[a]	25.18±0.00[b]	21.48±0.00[c]	12.74±0.00[d]	0.00

Duration of the experiment: 15 days; T: control; P10: batch of rats fed 10% protein diet; P5: batch of rats fed 5% protein diet; P0: batch of rats fed 0% protein diet (proteiprive diet); IW: initial weight; FW: final weight; IDM: Ingested dry matter; AWG: Average weight gain; FE: Feed efficiency; TPI: total protein intake; PE : protein efficiency and GEI: gross energy intake; the averages are followed by letters in super script (a, b, c, d, ...); averages with different letters on the same line are statistically different; P≤0.05: significant difference between the averages of the same line; ( ): number of rats; nd : not determied.

Table 4. Average value of serum metabolites of rats at the end of protein restriction

Serum parameters	Diet treatments				
	T (6)	P10 (6)	P5 (6)	P0 (6)	P Value
Urea (g/L)	0.26±0.05[a]	0.25±0.02[a]	0.18±0.04[b]	0.16±0.02[b]	0.05
U. acid. (mg/L)	43.00±3.10[a]	48.60±1.50[a]	48.20±1.83[a]	37.20±2.10[a]	0.53
Glucose (g/L)	0.74±0.02[a]	0.71±0.02[a]	1.3±0.42[a]	0.81±0.13[a]	0.22
Creat. (mg/L)	9.60±1.20[a]	9.60±0.60[a]	10.60±0.87[a]	7.20±0.58[a]	0.06
T. chol. (g/L)	1.83±0.07[a]	1.90±0.06[a]	1.85± 0.06[a]	1.84±0.05[a]	0.87
HDL chol. (g/L)	0.38±0.02[a]	0.41±0.02[a]	0.42±0.02[a]	0.37±0.02[a]	0.25
Triglyc. (g/L)	0.69±0.12[a]	0.77±0.08[a]	0.75± 0.06[a]	0.78±0.05[a]	0.85
T. bili. (mg/L)	15.56±3.20[a]	13.00±5.50[a]	7.80±0.73[b]	7.50±2.20[b]	0.03
C. bili. (mg/L)	2.50±0.82[a]	2.10±0.63[a]	0.72±0.01[b]	0.84±0.59[b]	0.01
ALAT (UI/L)	56.40±14[a]	54.60±5.60[a]	26.40±3.30[b]	24.40±6.60[b]	0.01
ASAT (UI/L)	58.60±15[a]	55.40±11[a]	24.60±5.00[b]	23.48±7.20[b]	0.03

Duration of the experiment: 15 days; T: control; P10: batch of rats fed 10% protein diet; P5: batch of rats fed 5% protein diet; P0: batch of rats fed 0% protein diet; U. acid : Uric acid; Creat. : creatinine; T. chol. .: total cholesterol; HDL chol.: HDL cholesterol; Triglyc. : triglyceride; T. bili.: total bilirubin; C. bili.: conjugated bilirubin; ASAT: aspartate aminotransferase; ALT: alanine aminotransferase; the averages are followed by letters in super script (a, b, c, d, ...); averages with different letters are statistically different; P <0.05: significant difference between averages; ( ): number of rats.

and PO (7.50±2.20 mg/L) are lower than that of control rats (15.56±3.20 mg/L) (P=0.03). Mean conjugated bilirubin values of P10 (2.10±0.63 mg/L), P5 (0.72±0.01 mg/L) and PO (0.84±0.59 mg/L) are lower than controls ones (2.50±0.82 mg/L) (P=0.01). With regard to enzymes, the mean values of the ALAT activity of the P10 rats (54.60±5.60 IU/L), P5 (26.40±3.30 IU/L) and P0 (24.40±6, 60 IU/L) are lower than that of control rats (56.40±14 IU/L) (P=0.01). The mean values of the ASAT activity of the P10 rats (55.40±11.00 IU/L), P5 (24.60±5.00 IU/L) and P0 (23.48±7.20 IU/L) are lower than that of control rats (58.60±15.00 IU/L) (P=0.03). The progressive decrease in the protein content of the diets induces a significant reduction in concentration of urea, bilirubin and ALAT and ASAT enzyme activity ($P \leq 0.05$).

Effects of protein restitution on the nutritional characteristics of rats: Differences in the nutritional characteristics of rats tested with restricted protein 10% (P10), 5% (P5) or 0% (P0) and control rats are significant ($p < 0.01$), at the end of the protein recovery period (Table 5). The final weights (PF) of the P10 rats (81.91±7.60 g), P5 (63.99±5.70 g) and P0 (46.91±3.00 g) are lower than those of the control rats (99.33±8.50 g). Mean ingested dry matter (IDM) values for P10 rats (4.45±0.00 g), P5 rats (3.94±0.00 g) and P0 rats (3.11±0.00) g) are lower than that of the control rats (5.10±0.00 g). The body weight gains (BWG) of the control rats (0.14±0.49 g), P10 rats (0.19±0.25 g) and P5 rats (0.03±0.23 g) are lower than the body weight gain of P0 rats (0.38±0.25 g). The feed efficiency coefficients (FE) of the control rats (0.03±0.07) and the rats tested P10 (0.04±0.07) and P5 (0.01±0.06) are lower than that of the rats tested P0 (0.12±0.08). The average total protein intake (TPI) values of the rats tested P10 (0.89±0.00 g), P5 (0.79±0.00 g) and P0 (0.62±0.00 g) are statistically lower than that of control rats one (1.02±0.00 g). The values of the protein efficiency coefficients (PE) of the control rats (0.13±0.38) and the rats tested P10 (0.22±0.74) and P5 (0.03±0.40) are statistically lower than that of the rats tested P0 (0.61±0.25 g) ($P = 0.01$). Ingested gross energy (IGE) values decreases from control rats (21.65±0.00 Kcal) to P0 tested rats (13.21±0.00 Kcal), via P10 rats (18.89±0.00 Kcal) and P5 (16.73±0.00 Kcal). Restitution of protein-deficient animals is not sufficient to make up for the nutritional characteristics of control animals after 15 days.

Effects of protein restitution on mean values of serum metabolites in rats: Table 6 indicates that most values of biochemical metabolites do not undergo significant variation ($P>0.05$). This is the case of uric acid, glucose, total cholesterol and HDL cholesterol, triglycerides and total bilirubin ($P>0.05$). On the other hand, significant differences exist between the urea, creatinine, conjugated bilirubin and ALAT and ASAT values of the different batches of rats. Mean creatinine values in protein deficient rats P10 (11.20±0.55 mg/L), P5 (11.80±0.73 mg/L) and P0 (10.25±0.63 mg/L) L) are higher than that of the control rats (9.40±0.04 mg/L). Mean values of conjugated bilirubin in rats P10 (0.50±0.50 mg/L), P5 (1.28±0.77 mg/L) and P0 (1.86±0.77 mg/L) are lower than controls one (3.36±0.35 mg/L). The activity of ALAT enzymes in rats P10 (28.20±2.10 IU/L), P5 (25.20±6.20 IU/L) and P0 (18.00±2.4 IU/L) is lower than that of the control rats (30.80±2.90 IU/L). As for the ASAT enzymes, the rats in batches P10 (25.60±2.80 IU/L), P5 (24.60±4.20 IU/L) and P0

Table 5. Average value of nutritional characteristics of rats at the end of protein restitution

Criteria	Diet treatments				
	T (6)	P10 (6)	P5 (6)	P0 (6)	P value
IW (g)	96.27±5.72[a]	79.03±3.30[b]	63.61±5.70[c]	41.16±2.70[d]	0.00
FW (g)	99.33±8.50[a]	81.91±7.60[b]	63.99±5.70[c]	46.91±3.00[d]	0.00
DMI (g)	5.10±0.00[a]	4.45±0.00[b]	3.94±0.00[c]	3.11±0.00[c]	0.00
AWG (g)	0.14±0.49[b]	0.19±0.25[b]	0.03±0.23[c]	0.38±0.25[a]	0.00
FE	0.03±0.07[b]	0.04±0.07[b]	0.01±0.06[b]	0.12±0.08[a]	0.01
TPI (g)	1.02±0.00[a]	0.89±0.00[b]	0.79±0.00[c]	0.62±0.00[d]	0.00
PE	0.13±0.38[c]	0.22±0.74[b]	0.03±0.40[d]	0.61±0.25[a]	0.01
GEI (kcal)	21.65±0.00[a]	18.89±0.00[b]	16.73±0.00[b]	13.21±0.00[c]	0.00

Duration of the experiment: 15 days; T: control; P10: batch of rats fed 10% protein diet; P5: batch of rats fed 5% protein diet; P0: batch of rats fed 0% protein diet (proteinprive diet); IW: initial weight; FW: final weight; IDM: Ingested dry matter; AWG: Average weight gain; FE: Feed efficiency; TPI: total protein intake; PE : protein efficiency and GEI: gross energy intake; the averages are followed by letters in super script (a, b, c, d, ...); averages with different letters on the same line are statistically different; P≤0.05: significant difference between the averages of the same line; ( ): number of rats.

Table 6. Average value of serum metabolites of rats at the end of protein restitution

Serum parameters	Diet treatments				
	T (6)	P10 (6)	P5 (6)	P0 (6)	P value
Urea (g/L)	0.25±0.06[b]	0.32±0.02[ab]	0.35±0.03[a]	0.33±0.03[ab]	0.05
U. acid. (mg/L)	28.80±3.6[a]	34.60±3.2[a]	31.20±3.7[a]	32.75±3.0[a]	0.06
Glucose (g/L)	0.75±0.05[a]	0.75±0.05[a]	0.62±0.00[a]	0.65±0.02[a]	0.07
Creat. (mg/L)	9.40±0.04[b]	11.20±0.55[ab]	11.80±0.73[a]	10.25±0.63[ab]	0.05
T. chol. (g/L)	1.71±0.03[a]	1.80±0.07[a]	1.67±0.07[a]	1.78±0.04[a]	0.51
HDL chol. (g/L)	0.39±0.00[a]	0.38±0.03[a]	0.37±0.03[a]	0.38±0.02[a]	0.95
Triglyc. (g/L)	0.74±0.07[a]	0.99±0.11[a]	0.75±0.06[a]	0.67±0.11[a]	0.68
T. bili. (mg/L)	7.00±0.32[a]	7.40±0.4[a]	10.00±1.4[a]	8.75±1[a]	0.12
C. bili. (mg/L)	3.36±0.35[a]	0.50±0.50[d]	1.28±0.77[c]	1.86±0.77[b]	0.03
ALAT (UI/L)	30.80±2.90[a]	28.20±2.1[b]	25.20±6.2[b]	18.00±2.4[c]	0.01
ASAT (UI/L)	31.60±4.4[a]	25.60±2.8[b]	24.60±4.2[b]	21.25±3.8[b]	0.00

Duration of the experiment: 15 days; T: control; P10: batch of rats fed 10% protein diet; P5: batch of rats fed 5% protein diet; P0: batch of rats fed 0% protein diet; U. acid : Uric acid; Creat. : creatinine; T. chol. .: total cholesterol; HDL chol.: HDL cholesterol; Triglyc. : triglyceride; T. bili.: total bilirubin; C. bili.: conjugated bilirubin; ASAT: aspartate aminotransferase; ALAT alanine aminotransferase; the averages are followed by letters in super script (a, b, c, d, ...); averages with different letters are statistically different; P <0.05: significant difference between averages; ( ): number of rats.

(21.25±3.80 IU/L) have a lower activity than that of control rats (31.60±4.40 IU/L). Protein restitution of malnourished rats causes an increase in serum urea and creatinine, and a decrease in conjugated bilirubin levels and enzyme activity (ALAT and ASAT).

Effects of fortified protein restitution on the nutritional characteristics of 0% protein restricted rats

Table 7 shows that at the end of the fortification recovery period, differences in the nutritional characteristics of the rats differed significantly (P<0.001). At the end of the fortified recovery period, the final weight (PF) of the fortified rats (P0F, 86.41±7.17 g) is greater than that of the P0 rats (50.60±1.89 g), but lower than that of the control rats (99.31±8.50 g). The value of the dry matter ingested (DMI) of the rats (P0F: 7.07±0.35 g) is greater than that of the P0 rats (3.54±0.00 g) and control rats (4.83±0.00 g). In fortifying diet rats(P0F), the weight gain (AWG) (3.17±0.85 g) was greater than those in the control rats (1.36±0.49 g) and rats subjected to restitution without fortification P0 (0,57±0,25 g). The value of the feed efficiency coefficient (FE) of the fortified rats (0.45±0.12) is higher than those of the control rats (0.28±0.07) and rats (P0) (0.16±0.08) subjected to the diet without fortification. Total protein ingested (TPI) values of fortified rats (P0F) (1.41±0.07 g) are higher than those of malnourished rats (P0) (0.71±0.06 g) and control rats (0.97±0.00 g). Protein Efficacy (PE) values of rats (P0F) (2.24±0.62) are higher than those of rats (P0) (0.81±0.42) and control rats (1.41±0.38).

The mean value of the gross ingested energy (GIE) of the rats subjected to the P0F regimens (30.02±1.5 Kcal) is higher than those of the control rats (20.52±0.00 kcal) and P0 rats (15.03±0.00 Kcal) (Table 7). The fortified restitution has the effect of significantly increasing the DMI and the AWG of the treated rats.

Effects of fortified protein restitution on the mean value of serum metabolites in rats with 0% protein restriction

Mean values for most serum metabolites do not vary significantly (P>0.05) (Table 8). This is the case for urea, uric acid, creatinine, total cholesterol, triglycerides, total bilirubin and ALAT and ASAT enzymes (P>0.05), except blood glucose, HDL cholesterol and conjugated bilirubin. Glycemia in rats fed fortification regimen (P0F: 1.03 ± 0.01 g/L) is higher than rats fed without fortification (P0: 0.69±0.02 g)/L) and that of the control rats (0.70±0.03 g/L). The concentration of HDL cholesterol in rats fed fortification (0.32±0.12 g/L) is lower (P=0.02) than in rats fed the P0 (0, 37±0.01 g/L) and that of the control rats (0.40±0.00 g/L). The concentration of the conjugated bilirubin of the rats subjected to the fortified restitution regimen (1.9±0.34 mg/L) is lower than those of rats (P0) (2.10±0.53 mg/L) and rats subjected to control diet (2.50± 0.82 mg/L). Fortified restitution of rats leads to an increase in blood glucose, a reduction in serum HDL-cholesterol and conjugated bilirubin.

Table 7. Average value of nutritional characteristics of 0 % protein restricted rats submitted to fortified restitution

Criteria	Diet treatments			
	T (6)	P0 (6)	P0F (6)	P Value
IW (g)	78.93±5.72[a]	42.05±1.57[b]	38.91±1.57[c]	0.00
FW (g)	99.31±8.50[a]	50.60±1.89[c]	86.41±7.17[b]	0.00
DMI (g)	4.83±0.00[b]	3.54±0.00[c]	7.07±0.35[a]	0.00
AWG (g)	1.36±0.49[b]	0.57±0.25[c]	3.17±0.85[a]	0.00
FE	0.28±0.07[b]	0.16±0.08[c]	0.45±0.12[a]	0.00
TPI (g)	0.97±0.00[b]	0.71±0.06[c]	1.41±0.07[a]	0.00
PE	1.41±0.38[b]	0.81±0.42[c]	2.24±0.62[a]	0.00
GEI (kcal)	20.52±0.00[b]	15.03±0.00[c]	30.02±1.50[a]	0.00

Duration of the experiment: 15 days; T: control; P0: batch of rats fed 0% protein diet (proteiprive diet); P0F: batch of rats fed 0% protein diet fortified; IW: initial weight; FW: final weight; IDM: Ingested dry matter; AWG: Average weight gain; FE: Feed efficiency; TPI: total protein intake; PE : protein efficiency and GEI: gross energy intake; the averages are followed by letters in super script (a, b, c, d, ...); averages with different letters on the same line are statistically different; P≤0.05: significant difference between the averages of the same line; ( ): number of rats.

Table 8. Average value of serum metabolites of 0 % protein restricted rats submitted to fortified restitution

Serum parameters	Diet treatments			
	T (6)	P0 (6)	P0F (6)	P Value
Urea (g/L)	0.27±0.02[a]	0.26±0.09[a]	0.30±0.04[a]	0.10
U. acid. (mg/L)	29.10±2.6[a]	31.71±3.02[a]	38.33±2.30[a]	0.26
Glucose (g/L)	0.70±0.03[b]	0.69±0.02[c]	1.03±0.01[a]	0.00
Creat. (mg/L)	8.94±0.41[a]	9.95±0.63[a]	9.70±1.50[a]	0.71
T. chol. (g/L)	1.71±0.03[a]	1.80±0.04[a]	1.70±0.013[a]	0.25
HDL chol. (g/L)	0.40±0.00[a]	0.37±0.01[a]	0.32±0.12[b]	0.02
Triglyc. (g/L)	0.71±0.11[a]	0.70±0.21[a]	0.79±0.07[a]	0.71
T. bili. (mg/L)	7.10±0.30[a]	8.74±1.45[a]	8.30±2.00[a]	0.45
C. bili. (mg/L)	2.50±0.82[a]	2.10±0.53[a]	1.90±0.34[b]	0.00
ALAT (UI/L)	29.80±2.51[a]	28.00±2.5[a]	31.00±9.50[a]	0.13
ASAT (UI/L)	30.60±3.40[a]	31.25±3.79[a]	29.67±10.00[a]	0.40

Duration of the experiment: 15 days; T: control; P0: batch of rats fed 0% protein diet; P0F: batch of rats fed 0% protein diet fortified; Urea : Uric acid; Creat. : creatinine; T. chol. .: total cholesterol; HDL chol.: HDL cholesterol; Triglyc. : triglyceride; T. bili.: total bilirubin; C. bili.: conjugated bilirubin; ASAT: aspartate aminotransferase; ALT: alanine aminotransferase; the averages are followed by letters in super script (a, b, c, d, ...); averages with different letters are statistically different; P <0.05: significant difference between averages; ( ): number of rats.

DISCUSSION

Effects of protein restriction and restitution with or without fortification on the nutritional characteristics of rats

After two weeks of treatment, the average values of the nutritional characteristics of the protein restricted rats were lower than those of the control rats. These results confirm those of Bennis Taleb (1997), whose Ph D. thesis focused on the effect of a diet deficient in proteins (8%) on the development of rats. Indeed, this doctoral student has shown that the low-protein diet induces a reduction in body weight, in both adult and young rats, compared to control rats. Similarly, in grasscutters, Yapi *et al.* (2013) have shown that a diet, below 6 g of digestible protein per MJ of digestible energy, is detrimental to the growth of animals. Amoikon *et al.* (2006) also showed that in pigs, growth is proportional to the increase in lysine levels in diets. Other work on pigs, during an initial phase of growth, allowed Henry *et al.* (1963) conclude that pigs tend to respond to a suboptimal intake of lysine or threonine, during the initial growth period, by increasing their feed intake per unit of metabolic weight. The results reported in this paper is in agreement with those of Handayani *et al.* (2017) who showed that the nutritional characteristics (ingested material, weight gain, energy intake) of malnourished rats (8.7% of proteins) are statistically lower than those of controls. Lac and Cnockaert (2008) have also shown that in rats of Wistar strain, a 2% casein diet stops the growth of animals, and conversely causes an increase in the volume of oxygen consumption. On the other hand, rehabilitation with a balanced diet (15% of casein) leads to an evolution of the values of these two parameters, comparable to that of controls of the same body weight. After two weeks of treatment, restitution results in a weak recovery of growth in experimental rats. The daily average values of the nutritional characteristics of the rats subjected to the protein restriction, followed by restitution, remain even lower than those of the control rats. These results confirm those of Amoikon *et al.* (2017). Indeed, through two experiments on dietary restriction in rats for 15 days and 30 days respectively, they concluded that with 10% or 20% of protein, growth in rats may be normal, provided that the amount of energy required is satisfactory. Similarly, the results obtained here are comparable to those of Bertrand (1991) who worked on cotton cake and rice flour. This author demonstrated that cottonseed cake and rice flour can replace soy flour in a rehabilitation diet for growing rats. Moreover, it has been demonstrated by Salles *et al.* (2014) that diets that contain fresh bee monofloral pollen improve muscle mass and metabolism in old undernourished rats. In these animals, the mitochondrial activity is depressed with food restriction, and is only improved by regeneration with fresh diets containing bee pollen. Proteins and energy are two fundamental factors (apart from micronutrients) of animal growth. However, their digestion may vary according to the stage of growth. The fortification of the proteiprive diet during the feed restitution causes a strong and very rapid recovery of the growth of the rats, tending to equal that of the controls in 15 days. The daily average values of the nutritional characteristics of the rats subjected to the protein restitution with fortification are higher than those of the malnourished rats subjected to a simple restitution. The results obtained do not confirm those of Azout *et al.* (1971) who report that the recovery of malnourished animals, using a balanced diet,

results in an immediate and significant acceleration of body growth, and at the same time, the synthesis of body proteins. According to Furuta and Murakami (2018), there is even a significant correlation between plasma amino acids (lysine, tryptophan, methionine and phenylalanine) and the body length of rats. In a similar experiment in the growing rat, Rérat *et al.* (1965), by improving the quality of the feed by antibiotic supplementation of diets, obtained an increase in consumption due in part to the improvement of nitrogen retention by antibiotics. Ultimately, improving the quality of the food by supplementing with amino acids, vitamins and trace elements, or by cleaning the environment of the digestive tract, leads to a rapid recovery of growth in animals malnourished. This conclusion is in agreement with the results obtained in this work with the fortification of the control diet by *Amin' total*. Indeed, this concentrate is often used in poultry breeding for the prevention and treatment of vitamins, trace elements and amino acids deficiencies. The results observed corroborate those of Henry and Rérat (1962) who showed that when the nitrogen requirement for maximum growth is not covered, the spontaneous consumption of energy is directly related to the quantity and the quality of the ingested proteins. It has even been reported by Munoz-Valverde *et al.* (2015) that malnutrition during gestation in rats can lead to reduced energy and changes in blood values in neonatal rats.

Effects of protein restriction and restitution with or without fortification on serum metabolites value in rats

The variation in the protein levels of the diets induces significant differences in the values of urea, bilirubins and the activity of ALAT and ASAT enzymes. Indeed, it is noticed a non-significant increase (P>0,05) of uremia in rats consuming for 15 days a fortified protein diet with *Amin' total*, compared to controls. This increase is, however, consistent with the work of Young *et al*. (2000) who reported that dietary protein intake stimulates urea production and excretion. The effect of malnutrition on enzymes has been demonstrated in other animal species. Corring *et al.* (1984), after an experience with a protein-free diet in pigs, showed that the amount of pancreatic protein does not change during this period of malnutrition. After two days of treatment, the specific activity related to chymotrypsin falls. While that of amylase and lipase drops slightly after six days. Malnutrition can affect other cellular and blood elements. According to Bennis Taleb (1997) and Molz *et al.* (2016), a diet low in protein results in a drop in DNA levels in the brain and liver of rats. Similarly, proteins and cholesterol levels are reduced in the brains of protein deficient rats. In addition, Pinheiro *et al.* (2013) showed that maternal protein restriction during pregnancy affects the expression and immunological localization of intestinal nutrient carriers in rats. According to Cortés-Barberena *et al.* (2013), moderate or severe malnutrition degrades spleen cell proliferation in the rat. The protein restitution causes, in the different batches of rats, significant changes in enzymatic activities (ALAT, ASAT). It does not affect the mean values of the biochemical parameters of the rats, after two weeks of experiment. According to Bennis Taleb (1997), recovery (restitution) can restore most of the biochemical values of protein deficient rats. The feeding of adult rats helps to restore blood volume and insulinemia. Fortified restitution causes a significant increase in blood glucose values and a decrease in HDL cholesterol, conjugated bilirubin in rats. These results support the hypothesis that aging-related malnutrition is often accompanied by numerous metabolic disturbances and dysfunctions, including the disruption of energy homeostasis (the installation of insulin resistance) and immune response disorders (Hamouda, 2015). This doctoral student concluded that the diets used in feeding can induce a very important reduction of the inflammatory status compared to that observed in the old rats. These results could be of interest in the prevention of pathologies associated with aging, related or not to undernutrition. According to Chambon-Savanovitch *et al.* (2001), a pancreatic extract may improve the feeding efficiency (growth) of malnourished rats. Similarly, Gautsch *et al.* (1999) demonstrated that growth hormone accentuates the recovery of somatic and skeletal muscle growth following chronic protein energy malnutrition.

Conclusion

Protein restriction causes in rats drop of the values of the nutritional characteristics of the rats. Protein restitution, without fortification causes a slight increase in the values of the nutritional characteristics. The fortification of a balanced diet results in a rapid restoration of nutritional characteristics and induces significant differences in the values of serum metabolites.

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