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EFFECTS OF PROTEIN-ENERGY RESTRICTION ON THE NUTRITIONAL STATUS OF GROWING RATS

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ABSTRACT

This study was undertaken to assess the effects of protein-energy restriction on nutritional indicators of growing rats. For this purpose, two experiments of 15 days and 30 days were carried out. For each one, 36 *Wistar* strain rats were divided into 6 groups of 6 rats each. Three groups of rats were fed *ad libitum*, and three other were subjected to the same diets with reduced daily feed intake. In addition, each experiment had one lot of rats subjected to a protein deprived diet. After 15 days of experiment, assessment of nutritional indicators of animals fed *ad libitum* showed that the decrease of body weight gain was proportional to protein content in diets. Similarly, whatever the protein content, energy restriction caused a significant deterioration in weight gain. After 30 days of experiment, weight loss in rats fed at will, and that of rats subjected to restricted diets were comparable to those of the first experiment. The results of both experiments indicated that 10 % or 20 % of casein protein appeared to be effective on the growth of young rats, provided that energy intake was sufficient.

INTRODUCTION

A study of FAO (1995) on malnutrition and nutrient deficiencies has classified protein-energy malnutrition (PEM) among the four major forms of malnutrition in the world. The prevalence of these status identified in Africa by FAO (1995) varies from one country to another. According to these estimates, the proportion of the population affected by chronic undernourishment increased by 5 % in sub-Saharan Africa since the 1970s (from 38 % to 43 %). Today, about 795 million people are undernourished worldwide (FAO, IFAD and WFP, 2015). But, because of regional population growth rate (3 % per year), the number of people affected has doubled. It is mostly, children who are affected by malnutrition. 165 million children have chronic PEM (UNICEF, 2013). 20 million children under 5 years suffer from severe acute malnutrition, with one million deaths per year. According to Aubry (2014), the consequences of chronic PEM in children under 5 years are stunted growth, delayed puberty, delayed psychomotor development; but above all, extreme sensitivity to infections and parasitic diseases.

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Protein-energy deficiency has very different effects on growth in animals and humans (Beck *et al.,* 1982; Fau *et al.,* 2006). The relationship between energy need and nitrogen need has been the subject of many studies (Gidenne and Lebas, 2005; Gidenne *et al.,* 2009a,b; Gidenne *et al.,* 2010). These studies showed that a lower energy intake provokes a loss of protein in adults and reduced growth in young children (Jacob, 1991). Consequently, the search for solutions to these problems related to PEM is still relevant. This study is therefore undertaken to evaluate the impact of protein-energy restriction on growing rats.

MATERIALS AND METHODS

Materials

Growing male rats of *Wistar* strain are used as biological material. Animals are weaned at the age of 4 weeks and have an average weight of 60±0.2 g at the beginning of the experiments. The animals are housed in individual cages. These cages were arranged on racks in stainless steel stored in an air conditioned room where the mean temperature and relative humidity recorded by a thermohygrograph "Casella" are 22 °C and 70 %, respectively. A period of 12 hours, alternating light and dark, is imposed. The diet (Table 1) is composed of vitamin mixtures (UAR), mineral mixtures (UAR), agar agar (Merck), cottonseed oil (TRITURAF, Ivory Coast), corn starch (Merck) and white sugar (Sucraf, Ivory Coast). Animals and feed ingredients are weighed on a scale of precision (Sartorius, Germany).

Methods

The first experiment lasts 15 days and the second 30 days. Each one is preceded by an adaptation of 8 days during which the animals receive a standard diet from commercial food for rabbits (IVOGRAIN, Abidjan). At the beginning of each test, the animals are divided into 7 groups. Three groups designated C20, C10, and C4 receiving respectively 20 %, 10 % and 4 % of casein in their diets, eating at will. And the other three designated C20R, C10R, and C4R fed with the same diets, but their daily feed intake is reduced by half, compared to that of animals fed *ad libitum*. And finally, a group of rats designated C0 receiving *ad libitum* a protein deprived diet.

Table 1. Composition of treatments (kg of DM)

Ingrédients (g of DM)	C20	C10	C4	C0
Casein	200	100	40	0
Corn starch	0	0	0	650
Cane sugar	200	200	200	200
Cottonseed oil	80	80	80	80
Agar agar (Cellulose)	10	10	10	10
Total dry matter (TDM)	1000	1000	1000	1000
Crude energy (Kcal. kg^{-1} DM)	4120	4120	4120	4120

Source : Hawrylewicz *et al.* (1982) et Alvarez *et al.* (1956) ;
UAR 200 et UAR 205 B (Villemoisson, 91360, Epinay/S/Orge, France) ;
Crude energy is calculated
according to values of consumption of different nutrients on the basis of :4 kcal for 1 g of protein,
4 kcal for 1 g of carbohydrates and 9 kcal for 1 g of lipids. Inclusion rates of casein : 20 % (C20) ; 10 % (C10) ;
4 % (C4) ; 0 % (C0) ; Energy restriction (50 %) diets are named C20R, C10R, C4R
Reduction rate of energy (50 %) : (C20R) ; (C10R) ; (C4R).

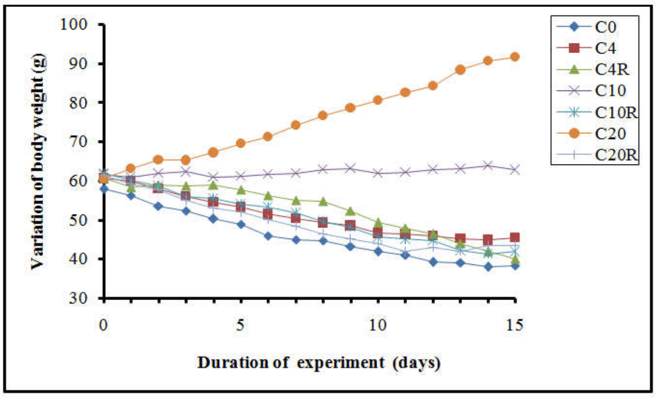
Statistical Analyses

A software "Statistica 7.1" is used for statistical analyses. Means with standard deviations are calculated. Comparison of the means is performed with the analysis of variances (ANOVA) for one factor, with $\alpha \leq 0.05$ as threshold. The differences are significant at the level of $P \leq 0.05$.

RESULTS AND DISCUSSION

Results

Variation of animals growth for 15 days : Figure 1 shows the growth curves of the animals subjected to different diets for 15 days (Exp. 1). The growth curves evolve with the inclusion rate of casein protein (C20, C10, C4). The animals of group C4 regularly lose weight. While those fed *ad libitum* with the C20 regimen have increased weight. Those rats subjected to the C10 diet have a slight increase of weight at the end of 15 days of the experiment. The rats subjected to protein deprived diet (C0) regularly lose weight. Similarly, all protein restriction batches (C20R, C10R, and C4R) lose weight from 1st to 15th day of experiment. It appears that the highest growth is observed with the C20 diet consumed at will, and the lowest one is observed with C4R regimen.



Inclusion rates of casein : 20 % (C20) ; 10 % (C10) ; 4 % (C4) ; 0 % (C0)
Reduction rate of energy (50 %) : (C20R) ; (C10R) ; (C4R)

Figure 1. Variation of growth of rats

Table 2 illustrates the influence of rationing on nutritional indicators of rats (Exp. 1). Body weight gains (BWG) of animals fed *ad libitum* (C20, C10, C4, C0), expressed in g/day/rat, are 2.08±0.20, 0.14±0.13, -1.01±0.4 and -1.30±0.20; respectively. Rats subjected to protein deprived diets, fed *ad libitum* lose weight from the beginning to the end of the experiment. In the 3 groups (C20, C10, C4, C0), dry matter intake (DMI) is 5.48±0.02, 5.16±0.03, 4.83±0.03, 3.62±0.13 g/day/rat and the amount of total protein intake (TPI) is 1.09±0.00, 0.51±0.00, 0.19±0.00, 0.00±0.00 g/day/rat. Growth efficiency (GE) and protein efficiency (PE) reports decrease proportionally. Rats subjected to protein deprived diets (CO) have TPI equal to zero. The body weight gains of animals subjected to restricted diets (C20R, C10R, C4R) are respectively -1.19±0.07, -1.33±0.05 and -1.37±0.08 g/day/rat. In the same rats, dry matter ingested (DMI) represents respectively 2.35±0.02, 2.30±0.03, 2.81±0.02 g/day/rat and TPI are 0.47±0.00, 0.22±0.00, 0.11±0.00 g/day/rat. The GE and the PE values are variable. Table 3 shows the comparison of average nutritional parameters in rats fed *ad libitum* or under energy restriction. Different contrasts show significant differences ($P \leq 0.05$) from one group to the other, in rats fed *ad libitum* or subjected the restricted diet.

Variation of animals growth for 30 days

Figure 2 illustrates the evolution of the body weight of rats for 30 days of experiment (Exp. 2). In this figure, there is a loss of body weight of rats, depending on the inclusion rate of casein protein (C20, C10, C4) in diets. Under energy restriction (50 %), it's shown the same trend of body weight in rats (C20R, C10R, C4R). The evolution of the curves in Figures 1 and 2 are similar. However, from the 25th day, animals in the group C20R loose more weight than the animals of group C10R. Nevertheless, it appears that the highest growth is observed with the C20 diet *(ad libitum),* and the lowest growth is illustrated by the C4R regimen. The rats subjected to deprived protein diet (C0) have a quantity of protein ingested (TPI) equal to zero, due to lack of casein in the constitution of the scheme (Table 4). Table 4 illustrates the influence of rationing on nutritional indicators of rats after 30 days of experiment (Exp. 2). Body weight gains (BWG) of animals fed *ad libitum* (C20, C10, C4, C0), expressed in g/day/rat, is respectively 2.43±0.06, 0.51±0.03, -0.65±0.03, -1.02±0.04. Dry matter intake (DMI) is 5.70±0.01, 4.22±0.01, 3.99±0.02, 2.25±0.04 g/day/rat, respectively.

Table 2. Growth parameters during 15 days

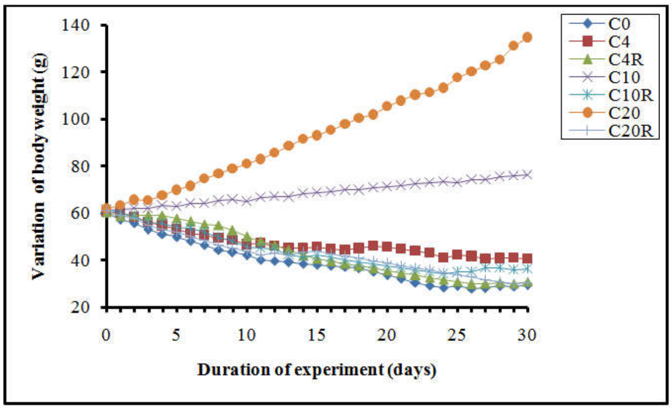
Criteria	Treatments						
	C20	C10	C4	C0	C20R	C10R	C4R
IW (g/j/rat)	60,58±1,83	61,75±1,16	60,55±0,75	57,86±2,13	61,33±0,58	61,92±0,67	60,63±0,52
FW (g/j/rat)	91,74±1,84	62,77±1,15	45,46±0,69	38,33±1,27	43,49±0,76	41,92±1,07	40,13±0,74
IDM (g)	5,48±0,02	5,16±0,03	4,83±0,03	3,62±0,13	2,35±0,02	2,30±0,03	2,81±0,02
BWG(g/j/rat)	2,08±0,20	0,14±0,13	-1,01±0,4	-1,30±0,20	-1,19±0,07	-1,33±0,05	-1,37±0,08
GE	0,38±0,04	0,03±0,03	-0,21±0,01	-0,36±0,06	-0,51±0,03	-0,58±0,02	-0,49±0,03
IP (g)	1,09±0,00	0,51±0,00	0,19±0,00	0,00±0,00	0,47±0,00	0,22±0,00	0,11±0,00
ICE (kcal.kg^{-1})	24,15±0,15	22,46±0,35	21,24±0,13	15,91±0,56	10,35±0,11	10,10±0,11	12,37±0,10

Inclusion rates of casein : 20 % (C20) ; 10 % (C10) ; 4 % (C4) ; 0 % (C0)
Reduction rate of energy (50 %) : (C20R) ; (C10R) ; (C4R) ;ICE: Ingested crude energy
IW : Initial weight; FW : Final weight; IDM: Ingested dry matter; BWG : Body weight gain;
GE : Growth efficiency ; IP : Ingested proteins ;

Table 3. Statistical analyses of nutritional parameters during 15 days

T / NP	C20 / C10	C20 / C4	C10 / C4	C20R / C10R	C20R / C4R	C10R / C4R
IDM	P<0,0001	P<0,0001	P<0,0001	P<0,0001	P<0,0001	P<0,0001
BWG	P<0,0001	P<0,0001	P<0,0001	P=0,0165	P=0,0047	P=0,5821
IP	P<0,0001	P<0,0001	P<0,0001	P<0,0001	P<0,0001	P<0,0001
ICE	P<0,0001	P<0,0001	P<0,0001	P<0,0001	P<0,0001	P<0,0001

P ≤ 0,05 : Significant différence ; P>0,05 : non significant différence
Inclusion rates of casein : 20 % (C20) ; 10 % (C10) ; 4 % (C4) ; 0 % (C0)
Reduction rate of energy (50 %) : (C20R) ; (C10R) ; (C4R)
T : Treatments ; NP : Nutritional parameters ; IDM : Ingested dry matter ; BWG: Body weight gain ;
GE : Growth efficiency ; IP : Ingested proteins ; ICE: Ingested crude Energy



Inclusion rates of casein : 20 % (C20) ; 10 % (C10) ; 4 % (C4) ; 0 % (C0)
Reduction rate of energy (50 %) : (C20R) ; (C10R) ; (C4R)

Figure 2. Variation of growth of rats

Table 4. Growth parameters of rats during 30 days

Criteria	Treatments						
	C20	C10	C4	C0	C20R	C10R	C4R
IW(g)	61,85±0,63	61,20±0,18	60,48±0,20	60,14±1,70	61,33±0,25	61,74±0,58	60,50±0,41
FW(g)	134,75±1,61	76,50±0,96	40,75±1,01	29,49±0,76	30,50±1,05	36,29±0,50	30,98±1,40
IDM(g)	5,70±0,01	4,22±0,01	3,99±0,02	2,25±0,04	1,51±0,01	1,52±0,01	1,83±0,01
BWG(g)	2,43±0,06	0,51±0,03	-0,65±0,03	-1,02±0,04	-1,02±0,04	-0,84±0,01	-0,98±0,06
GE	0,42±0,01	0,12±0,00	-0,16±0,00	-0,45±0,02	-0,67±0,02	-0,55±0,01	-0,53±0,03
IP(g)	1,14±0,00	0,42±0,00	0,15±0,00	0,00±0,00	0,30±0,00	0,15±0,00	0,07±0,00
ICE (kcal.kg^{-1})	25,11±0,05	18,60±0,05	17,58±0,09	9,90±0,18	6,65±0,05	6,69±0,06	8,05±0,05

Inclusion rates of casein : 20 % (C20) ; 10 % (C10) ; 4 % (C4) ; 0 % (C0)
Reduction rate of energy (50 %) : (C20R) ; (C10R) ; (C4R) ;ICP: Ingested crude energy
IW : Initial weight; FW : Final weight; IDM: Ingested dry matter; BWG : Body weight gain;
IP : Ingested proteins ; GE: growth efficiency

Table 5. Statistical analyses of nutritional parameters during 30 days

T / NP	C20 / C10	C20 / C4	C10/ C4	C20R / C10R	C20R / C4R	C10R /C4R
IDM	P<0,0001	P<0,0001	P<0,0001	0,7686	P<0,0001	P<0,0001
BWG	P<0,0001	P<0,0001	P<0,0001	P<0,0001	P<0,0001	P<0,0001
IP	P<0,0001	P<0,0001	P<0,0001	P<0,0001	P<0,0001	P<0,0001
ICE	P<0,0001	P<0,0001	P<0,0001	P<0,0001	P<0,0001	P<0,0001

P ≤ 0,05 : significant différence ; P >0,05 : non significant différence
Inclusion rates of casein : 20 % (C20) ; 10 % (C10) ; 4 % (C4) ; 0 % (C0)
Reduction rate of energy (50 %) : (C20R) ; (C10R) ; (C4R)
T : Treatments ; NP : Nutritional parameters ; IDM : Ingested dry matter ; BWG: Body weight gain ;
IP : Ingested protein ; ICE: Ingested crude energy

The amount of total protein ingested (TPI), *ad libitum,* is 1.14±0.00, 0.42±0.00, 0.15±0.00, 0.00±0.00 g/day/rat. The GE and PE values decrease proportionally among the 4 batches fed *ad libitum.* The rats subjected to protein deprived diet (C0) have TPI equal to zero. Rats undergoing energy restriction (50 %) (C20R, C10R, C4R), lose body weight (-1.02±0, 04, -0.84±0.01, -0.98±0.06 g/day/rat) proportionally to the rate of inclusion of casein protein in diets. DMI goes up (1.51±0.01, 1.52±0.01, 1.83±0.01 g/day/rat, respectively) and TPI goes down (0.30±0,00, 0.15±0.00, 0.07±0.00 g/day/rat, respectively). However, GE and PE decrease, depending on the protein inclusion rate in diet. Table 5 shows the comparison of average nutritional parameters in rats fed *ad libitum* or under energy restriction. Results show significant differences (p≤0.05) from one lot to another in rats fed *ad libitum* or subjected to restricted diet.

DISCUSSION

Results of the two experiments show that the decrease of body weight is due to changes in protein and energy rate plans. These variations affect the consumption of dry matter, and therefore, energy intake. For example, the C20 diet is better used as a dry matter intake (MSI), followed by diets C10 and C4. The relationship between the protein level in diets and consumption of dry matter confirms the results obtained by Fockdey and Arnould (1972), in growing chicks. Concerning energy restriction diets, the highest value of IDM is achieved in batches fed with diet containing 4 % (C4R) of protein. Decreasing weight of rats is proportional to the levels of animals ration. These results have already been observed by Gidenne *et al.* (2003). They are similar to those of Perrier (1998), Boisot *et al.* (2003), and Foubert *et al.* (2008). These authors reported that for higher levels of dietary restriction (30%, 40% and 50%), body weights are then reduced by 14 % to 20%. Weight gain observed with the C20 diet could indicate a steady development of cellular metabolism with an efficient synthesis of lean material in rats under this regimen. The significant differences between average values of different parameters can be explained by a higher weight gain induced by diet C20. This performance obtained with 20 % of the casein content is explained by the high levels of this protein in the diet ingested *ad libitum.* Moreover, Rahman *et al.* (2005), Zannou-Tchoko (2005), Bouafou *et al.* (2007), Amoikon *et al.* (2013) showed the importance of the quality of protein served to growing rats. Weight loss induced by the regimen with 4 % protein in *ad libitum* fed batches and all energy restriction diets, is the result of insufficient energy and dietary protein intake. In humans, energy and protein deficiencies are the causes of marasmus or kwashiorkor, or both (Aouehougon, 2007). Dietary restriction influences the use of dietary protein through a decrease in the synthesis of nitrogen-containing tissues, including muscle tissues (Rérat *et al.,* 1971).

Diets seem more effective when the protein content of the diet is higher. These results have already been observed by Henry *et al.* (1965) and Jahanian and Edriss (2015). However, it is necessary to associate both protein and energy requirements (Abbas *et al.*, 2015). Indeed, energy restriction alters much more animal growth of C20R than other rats (C10R and C4R). Deprived of its main energy substrate that is carbohydrates, the body will draw energy from lipids and proteins. Weight loss following ingestion of restricted diets shows that energy is a key factor in the use of diets. It is well established that when the energy requirement is not fully covered, most of the protein is converted to energy (Raimbault, 1980). In this case, proteins are oxidized and amino acids resulted are converted into glucose to meet the energy deficit (Butter *et al.,* 1989; Sugiyama *et al.,* 1991 and 1992). The protein turnover decreases because proteolysis seems higher than protein synthesis, which induces a negative protein balance (Sauvant *et al.,* 2004). Therefore, the limiting factor in muscle synthesis appears to be energy. Proteins are not meant to provide energy to the body, but rather participate in its construction, restoration, replacement of dead cells, production of enzymes and hormones. In addition to excessive weight loss, edema were observed on the legs and on the tails of rats subjected to energy restriction diets with low protein content (C4R). These symptoms indicating a clinical picture similar to that of Kwashiorkor is a consequence of protein-energy malnutrition in children. These results confirm the role of protein and energy need in the prevalence of protein-energy malnutrition. Growth is the result of the overall functioning of the whole body. Therefore, it is likely that its disruption comes from organ failure due to protein-energy malnutrition.

Conclusion

At the end of this study, it appears that growing rats are very sensitive to the balance of protein and energy in the diet. The above results show that an imbalance in the energy and protein intake jeopardizes growth and provokes protein-energy malnutrition. These results indicate that a diet containing 20 % or 10 % of casein protein appears to be effective on the growth of young rats, provided that energy intake is sufficient. The only satisfaction of protein requirement is not enough to prevent nutritional diseases.

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