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Original

Flaxseed and its contribution to body growth and brain of Wistar rats during chidhood and adolescence

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Abstract

Objectives: **To evaluate the effect of flaxseed upon body growth and brain of rats.**

Methods: **Experimental phase lasted 52 days, using 42 Wistar rats which were divided into four groups: Control (CG, n = 12), 10% casein diet; Flaxseed (GL, n = 12), 10% flaxseed diet plus casein; Modified Control (GCM, n = 12), 10% casein diet with changes in lipid and fiber comparable to GL; Non-protein (GA, n = 6), diet without protein. Considering food intake, protein intake and weight variation, the Protein Efficiency Ratio (PER) was calculated. Net Protein Retention (NPR) and the Food Efficiency Ratio (CEA) were also determined relative to brain weight at 30 days of life (M30) and at 52 days (M52). Analysis of Variance (ANOVA), Test of Sheffé and post-test of Bonferroni were used, p ≤ 0.05.**

Results: **GL had lower food intake, protein and weight variation than GC, but had higher values than GCM. Concerning PER, GL was lower than GC and similar to the GCM, as well as CEA. As for NPR, GL had lower values than the CG and GCM. At M30, GL was superior to GC in relation to brain weight. Likewise, the same was observed at M52.**

Conclusion: **Flaxseed promoted adequate growth and better brain development in animals, which might be explained by increased incorporation of omega-3 into these tissues.**

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Key words: *Flaxseed. Casein. Rats. Weight variation. Omega-3. Brain.*

LINAZA Y SU CONTRIBUCIÓN AL CRECIMIENTO DEL CUERPO Y EL CEREBRO DE RATAS WISTAR DURANTE LA INFANCIA Y LA ADOLESCENCIA

Resumen

Objetivos: **Evaluar el efecto de la linaza en el crecimiento corporal y del cerebro de ratones.**

Métodos: **El estudio experimental duró 52 días, utilizando 42 ratones Wistar, que fueron divididos en cuatro grupos: Control (GC, n = 12), dieta con 10% de caseína; Linaza (GL, n = 12), 10% de la dieta de linaza adicionada de caseína ; Control Modificado (GCM n = 12), 10% de caseína con los cambios en los lípidos y fibra comparable a GL, Sin proteína (GA, n = 6), dieta sin proteínas. Fueron controlados la ingesta de alimentos, la ingesta de proteínas y la variación de peso, fueron calculados el Índice de Eficiencia Proteica (PER), la retención proteica neta (NPR) y el Índice de Eficiencia de Alimentación (CEA). Se determinó también el peso cerebral relativo a los 30 días de vida (M30) y 52 días (M52). Fue utilizado ANOVA, prueba de Sheffé y coeficiente de Bonferroni, con p≤ 0,05.**

Resultados: **GL tuvo menor consumo de alimentos, proteínas y variación del peso sobre GC, pero tuvo valores más altos que GCM. Con relación a PER, GL fue inferior a GC y similar a GCM, así como en el CEA. GL tuvo valores menores valores de NPR que GC y GCM. En M30, GL fue superior a GC en relación al peso relativo del cerebro. Lo mismo se observó en M52.**

Conclusiones: **La linaza promueve un crecimiento adecuado y un mejor desarrollo cerebral en los animales, lo que podría explicarse por una mayor incorporación de ácidos grasos omega-3 en estos tejidos.**

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Palabras clave: *Linaza. Caseína. Ratones. Variación del peso. Omega-3. Cerebro.*

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Abbreviations

GC = Control.
GL = Flaxseed.
GCM = Modified Control.
GA = Non-protein.
PER = Protein Efficiency Ratio.
NPR = Net Protein Retention.
CEA = Food Efficiency Ratio.
M30 = 30 days of life.
M52 = 52 days of life.
PUFAs = Polyunsaturated Fatty Acids.
EFAs = Essential Fatty Acids.
ALA = Alpha Linolenic Acid.
EPA = Eicosapentaenoic Acid.
DHA = Docosahexaenoic Acid.
SBCAL = Brazilian Society of Animal Science Laboratory.
Labne = Laboratório de Nutrição Experimental.
AIN-93 = American Institute of Nutrition.
ANOVA = Analysis of Variance.
Σn-3 = Sum of omega-3

Introduction

Physical growth is characterized by the sum of cellular, biological, biochemical and morphological events, whose interaction is accomplished by genetic background and influenced by the environment and adequate nutrition, an essential factor to ensure normal growth.[1]

As far as nutrients are concerned, adequate supply of proteins, carbohydrates and lipids is essential for the maintenance, growth and reproduction of animal species.[2]

During growth spur and onset of myelination, there is a larger saturated and unsaturated long-chain essential's accumulation for the progressive maturation of the brain tissue.[3]

Polyunsaturated fatty acids (PUFAs) of n-3 series, found in the brain and retina, contribute to the process of myelination, development of visual function, psychomotor development and various aspects of neural function in relation to behavior. Moreover, its importance in the development of central nervous system of infants is well documented.[4]

Studies show that nutrition in utero and at postnatal periods, with specific fatty acids, have a marked effect at older ages of life, demonstrating the importance of an adequate supply of essential fatty acids (EFAs) during pregnancy, lactation and childhood, which are the most vulnerable periods for brain development.[5]

At the same time, flaxseed has been described as presenting a high content of alpha-linolenic acid (C18:3n-3, ALA), ranging from 44.6 to 51.5% of total fatty acids, and this is a polyunsaturated fatty acid that can be converted to long chain *omega-3* in the body: eicosapentaenoic acid (C20:5n-3, EPA) and docosahexaenoic acid (C22:6n-3, DHA). Approximately 20% of alpha-linolenic acid contained in flaxseed is converted in the body into EPA and DHA.[6]

Phospholipid membranes of the brain are highly enriched with DHA, which can affect brain function and behavior and also promote fetal development.[7]

Taking these facts into account, the study was designed to evaluate the effect of flaxseed consumption on body growth and brain development of rats from childhood to adolescence.

Material and methods

Ethics

The experiment was conducted in accordance with provisions of the Brazilian Society of Animal Science Laboratory (SBCAL) and was approved by the Ethics committee in Research of Hospital Universitário Antônio Pedro, UFF, opinion number 188/06.

Animals

42 Wistar rats, 21 days old, newly weaned and average weight of 42g were used. They were obtained from the *Experimental Nutrition Laboratory* (Labne), Department of Nutrition and Dietetics, Nutrition College, *Universidade Federal Fluminense, Niterói, RJ, Brazil.*

Experimental Design

Animals were divided into four groups: Control (GC), consisting of 12 animals fed a casein based diet; Flaxseed (GL), consisting of 12 animals, fed onto a flaxseed based diet plus 5.43% of casein; Modified Control (GCM), consisting of 12 animals fed casein based diet, but with higher concentrations of oil and fiber, aiming to achieve the levels of fiber and lipids of the diets based on flaxseed; and Non-protein (GA), consisting of 6 animals fed protein free diet and used for completion of the biological Net Protein Retention (NPR).[8]

Pair feeding was performed in GCM with the GL, aiming to offer the same amount of diet consumed by the GL to GCM.

All diets were isonitrogenous, with 10% protein in their composition, balanced and prepared in accordance with the recommendations of the American Institute of Nutrition-AIN-93,[9] as shown in table I.

Pups were taken from mothers who received the experimental diets (casein, modified casein, and flaxseed) during gestation and lactation. At 21 days old pups were separated from their mothers and kept in individual cages, with constant temperature (22 ± 2°C) and controlled lighting, with light-dark cycle 12/12 h. Water was offered *ad libitum* and food intake was con-

Table I
Formulation of diets (g/100 g food) with 10% protein

Components	*Control*	*Flaxseed*	*Modified control*	*Non-protein*
Casein*	11,76	5,9	11,76	–
Flaxseed†	–	25,0	–	–
Starch‡	61,19	54,0	55,49	73,25
Sugar§	10,0	10,0	10,0	10,0
Mineral mix*	3,5	3,5	3,5	3,5
Vitamin mix*	1,0	1,0	1,0	1,0
Soybean oil‖	7,0	–	11,0	7,0
Cellulose**	5,0	–	6,7	5,0
B-choline*	0,25	0,25	0,25	0,25
Cystine*	0,3	0,3	0,3	0,3
Total	100	100	100	100

*Rhosther Industria e Comércio LTDA, †ArmaZem LTDA, ‡Maisena, §União, ‖Liza®, **Macrocel®.

trolled, being the difference between the offered and leftover food. Body weight of each animal was evaluated 3 times a week, throughout the experiment, which lasted 52 days.

Biological analysis

Protein Efficiency Ratio (PER) was used so as to evaluate biological protein quality. This method consists of the ratio between the weight gain of animals and protein consumption of between zero and 28 days.[8]

Food Efficiency Ratio (CEA), consider the weight gain of animals between day zero (after weaning) and day 28 as well as the cumulative consumption of food until the 28th day.[8]

Net Protein Ratio (NPR) adds to body weight gain in protein diet group, whereas it adds to weight loss in the group fed onto the protein free diet.[8]

Brains

Brains were obtained in animals at 30 days old and 52 days old as these points represents approximately the beginning and the end of body growth, making it possible to verify an association between body growth and brain development in rats.

At 30 days of life, pups (M30) from GC, GL and GCM (6 animals each group) were sacrificed aiming at brain collection, and the remaining 18 animals, also 6 animals for each group (GC, GL and GCM) were sacrificed at 52 days of life (M52).

In these two moments of sacrifice, the animals were sacrificed by guillotine and the brains were removed with the aid of forceps and scissors and weighed on an analytical balance (Bosch S 2000).

After this procedure, we performed the calculation of relative brain weight, where we used the weight of the animal's brain, divided by body weight, and the result was multiplied by 100, which is described as a percentage value (%).

Statistical analysis

The data were presented as mean and standard deviation. The results obtained was applied to analysis of variance (ANOVA), with $p \leq 0,05$. When detected statistically significant variable, test was applied for two averages *Sheffé* by the *Coefficient of Bonferroni* for multiple comparisons. Statistical analysis was performed by *Software Statgraphics Plus* 6.0.

Results

As for food intake of animals (table II), GL had lower intake than GC ($p \leq 0,05$), but higher than GCM ($p \leq 0,05$). The same was observed regarding protein intake and body weight gain of animals (table II).

When the protein quality was evaluated through PER (fig. 1a), GL had lower value than GC ($p \leq 0,05$), but was similar to GCM. The same was observed in values of CEA (fig. 1b).

Table II
Food intake, protein intake and weight variation of animals

Groups	*Food intake (g)*	*Protein intake (g)*	*Weight variation (g)*
*GL	333,86 ± 4,89[a]	34,38 ± 0,50[a]	100,50 ± 2,20[a]
†GC	450,47 ± 6,30[b]	46,41 ± 0,64[b]	174,58 ± 3,88[b]
‡GCM	276,75 ± 6,12[c]	28,50 ± 0,63[c]	86,83 ± 2,79[c]

Different superscript letters in the same column indicate significant difference ($p \leq 0.05$).
*Flaxseed, †Control; ‡Modified control (n = 12/group).

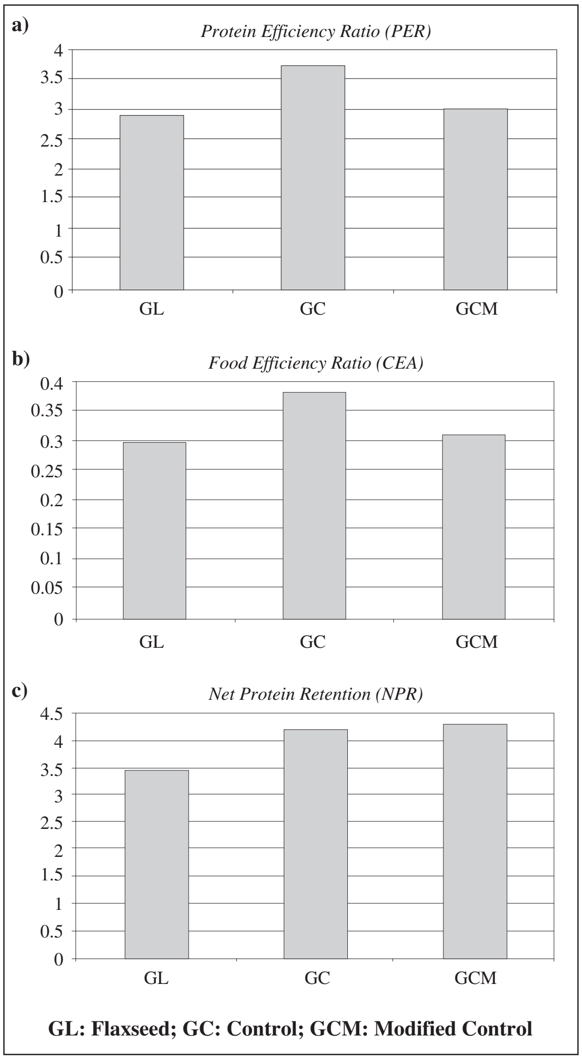

Fig. 1.—Rats body growth.

Considering the values of NPR (fig. 1c), it was perceived that the GL remained below ($p \leq 0,05$) the GC, also proved to be smaller ($p \leq 0,05$) than GCM.

Taking into account the averages for the relative brain weight in M30 (table III), it was noted that GL was higher than GC ($p \leq 0,05$) and similar to GCM.

However, it was observed that GL was higher than GC ($p \leq 0,05$) and GCM concerning the relative brain weight of animals at M52 (table III).

Discussion

Growth is a dynamic and continuous process that occurs from conception until the end of life and is expressed by the increase in body size. The use of protein during growth is maximized and more efficient with lower levels of dietary protein than is required to maintain minimum organic activities.[10]

In this study, food intake of animals fed on flaxseed was below the GC and higher than GCM, reflecting a lower protein intake by GL when compared to GC and a higher consumption when compared to the GCM. This inferiority in relation to GC can be justified by the high-fiber diet and fats present in flaxseed.

Table III
Relative cerebral weight (RCW) thoughout the experiment

Groups	*M30* *RCW (%)*	*M52* *RCW (%)*
*GL	2,89 ± 0,16[a]	1,41 ± 0,08[a]
†GC	2,15 ± 0,12[b]	1,09 ± 0,23[b]
‡GCM	3,16 ± 0,12[a]	1,34 ± 0,07[b]

Different superscript letters in the same column indicate significant difference ($p \leq 0.05$).
*Flaxseed, †Control, ‡ Modified control (n = 12/group).

Fibers are found in large quantities in flaxseed (28%) which are soluble and insoluble, causing animals to have a greater satiety, thus leading to lower consumption. Moreover, the amount of calories present in 100 grams of flaxseed is 396 kilocalories, distributed in 109 kcal of protein, and 287 kcal of lipid, corresponding to 31g lipid and 24.40g protein per 100 grams.[11]

By analyzing the changes in body weight, it was noted that GL presented lower food and protein intake than GC, and this superiority of GC can be attributed not only to increased to higher amount of intake, but also to the high biological value of casein. Moreover, GL also showed reduced food and protein intake when compared to GCM and GL presented superior weight variation when compared to this group. Besides quality, the quantity of protein consumed directly interferes with a larger body growth, accompanied by greater changes in body weight.[12]

Another study found values of initial body weight for animals under the same conditions of creation around 49,50 ± 5,11g (*Wistar* rats). Regarding final weight, the values were 147,52g ± 22,72, and both approached those found in the control group of experiment.[13]

When analyzing a diet containing a mixture of vegetable and animal proteins (as offered to GL), and comparing it to a diet based on casein (as offered to GC), it was found greater weight gain in animals fed exclusively on animal protein diet, which agrees with the present study.[14]

Most animal protein has good digestibility, resulting in an effective absorption of amino acids. The vegetable proteins are generally poorly digestible, which justifies the lowest values found in the GL when compared to GC.[15]

One method for assessment of growth and protein utilization used in this experiment was PER. PER value above 2 is related to a good quality and high protein, and a PER value below 1.5 is associated with a low quality protein.[16] Thus, it can be stated that diet provided to GL is good, since this group exhibited a PER greater than 2.

However, previous studies which used the flaxseed as exclusive protein source, PER value was extremely low, below 1.5, then classify this seed as a protein source of low quality.[17]

Regarding CEA value, GL showed a lower rate when compared to GC, and this can be explained by its high fiber content, fat and anti-nutritional factors. GL showed a value statistically similar to GCM, which can be justified by the same concentration of fiber in the diets offered to both of them.

Some dietary components modify the use of protein such as fiber and phytates, which can affect the bioavailability of various nutrients.[18]

In another experiment, the results were similar to the present study, aimed to analyze the influence of the addition of an alternative food supplement upon growth and development of rats. This supplement was made up of a combination of meals, vegetable seeds and milk powder. It was observed low CEA value for this supplement (0.01 ± 0.04) when compared to GC.[19] This fact can be explained by the large amount of anti-nutritional factors present in the seeds and the amount of bran found in this supplement, which inhibit the absorption of protein.[20]

By analyzing the NPR values, it was found that GL was unable to match the groups fed with casein, showing that the flaxseed diet was made up of a protein of low biological value.

Concerning the use of diets with defatted soybean meal, it was reported values ranging from 2.50 à 3.70, for NPR, lower than casein group, which showed a value of 4.70.[21] This finding is similar to this study.

In recent years, the amount of lipids supplied in the diet has drawn attention of many researchers, especially its effects upon children, because this is a determining factor in growth, visual and neural development, and health maintenance.[22]

The term "brain development" is often treated in literature as total growth or weight gain in the total brain. Thus, the brain weight is associated with its development and suggests that the incorporation of fatty into tissue affects brain development.[23]

By observing the averages for the relative brain weight of animals at day 30 of life (M30), was noted that the largest percentage of brain weight was on GL when compared to GC and it was observed homogeneity between GL and GCM.

This result of relative brain weight of GL can be attributed to large amounts of fatty acids from omega-3 family in flaxseed, which has 57% of omega-3 fatty acids in their composition,[24] besides this diet have been offered since the gestation and lactation of the pups until 52 days of their life (M52), which generated a greater aggregation of these fatty acids in brain tissue, thus increasing its weight due to a higher content of DHA absorbed by the brain during development of central nervous system.[25]

After comparing the brains of rat pups from mothers fed diets with or without essential fatty acids, it was found that brains of pups from mothers fed diets in the presence of essential fatty acids showed higher accumulation of DHA and sum of omega-3 (Σn-3).[26] Likewise, analyzing the relative brain weight of animals at M52, it was perceived that that GL was superior to both groups fed with casein diet.

Another study found a content of 24.4 ± 0.2 (%) of alpha-linolenic acid in the diet supplemented with flaxseed oil in its composition, while in the diet without flaxseed oil, the content of that fatty acid was 1.58 ± 0.03 (%). As for the composition of fatty acids in tilapia head subjected to this experiment, the values were 1.08 ± 0.12 (%) of alpha-linolenic acid in the treatment without flaxseed oil, and 3.80 ± 0.80 (%) of alpha-linolenic acid, after 30 days of treatment with flaxseed oil. On the other hand, in treatment without flaxseed oil was found a content of 0.20 ± 0.06 (%) of DHA and 30 days after treatment with flaxseed oil a content of 0.32 ± 0.04 (%) of DHA was found.[27] Furthermore, a study of diets with different proportions of flaxseed, rapeseed oil, sunflower oil and tallow, also observed that higher values of the series PUFAs omega-3 long-chain (EPA and DHA) were found in the diet with flaxseed.[28]

In addition, after the administration of a single dose of 20 mg of an oil (containing alpha-linolenic acid, omega-3 family, and linoleic acid, omega-6 family) in rats with 7 weeks of life, it was observed a higher concentration of DHA in the brain and spinal cord of these animals.[29] Beyond the observation that DHA is widely distributed in high concentration in central nervous system and thus plays a critical role throughout development of this system.[30] These facts confirm that from a diet in which there is presence of omega-3, DHA is obtained as one of its products and this is more expressed in the central nervous system.

Conclusion

Flaxseed diet promoted adequate growth in animals and a better brain growth, in addition to possibly have contributed to an increased omega-3 fatty acids incorporation to this tissue, suggesting a better development of the animals fed this diet both during childhood and adolescence.

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