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Evaluation of Protein Quality from Genetically Modified and Organic Soybean in two Consecutives Generations of Wistar Rats

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ABSTRACT

The aim of this study was to evaluate the cumulative effects of using genetically modified and organic soybean in two generations of rats. Two consecutive generations of 64 Wistar rats denominated F_0 and F_1 were used. The animals from each generation were divided into three groups (n=8) and fed chow made of organic soybean, genetically modified soybean and casein. The PER, NPR and CAE were determined. ANOVA was applied to the results. In both the generations, a statistically significant ($p<0.05$) difference was found between the experimental groups and the control group. The organic soybean supplement with L-cystine provided a better use of this protein in comparison to the genetically modified soybean protein, which demonstrated that the soybean maintained its protein use, although always inferior to casein. Thus, soybean should be used with caution during the growth stages because even with supplements, it remained inferior in relation to the casein group.

Key words: soybeans, *Wistar* rats, protein quality, generations

INTRODUCTION

Soybean has received a great deal of attention from the scientific community, especially due to its profile of nutrients. It has complex carbohydrates, protein, soluble and insoluble fibers, oligosaccharides and phytochemicals. Some authors describe the soybean as a rich source of proteins since its composition is comprised on average of 30 to 45% proteins, 20 to 25% carbohydrates and 15 to 25% lipids. It also contains minerals such as Iron (Fe), Zinc (Zn), Magnesium (Mg), Potassium (K), Calcium (Ca), Manganese (Mn), and Selenium (Se) and vitamins such as retinol, thiamine, riboflavin, pyridoxine and folic acid (Anderson et al., 1999).

Soybean is broadly used as a source of edible oil and protein for human and animal foods due to its high nutritional and functional values. Approximately 60% of the products processed in the food industry around the world contain ingredients derived from soybean (Soares et al., 2005). The composition of the soybean includes antinutrients that limit its use, such as lectins, oligosaccharides, phytates, saponins and protease inhibitors (Sant'ana et al., 2000; Barcelos et al., 1999; Miura et al., 2001; Monteiro et al., 2004; Vasconcelos et al., 2001). The protease inhibitors, such as the Kuntz Trypsin Inhibitor (KTI) and the Bowman-Birk (BBI) trypsin and chemotrypsin inhibitor, inhibit proteolytic enzymes, which consequently reduces the food protein digestion, causing a reduction in the animal's weight gain

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and growth (Miura et al., 2001; Monteiro et al., 2004). This can lead to hypertrophy of the pancreas due to the increased need for gastric, trypsin and chemotrypsin enzyme production to digest the proteins (Vasconcelos et al., 2001). At present, the thermal treatment has been used as a means to alleviate the antinutritional factors and improve the nutritional use of soybeans (Sgarbieri et al., 1982). It also helped improving the development of genetically modified varieties lacking these inhibitors (Vasconcelos et al., 2001; Carpentieri-Pípolo et al., 2000).

The improvement in digestibility with the treatment is attributed not only to the reduction in antinutritional factor activity but also to the structural alterations in soybean protein, since these have a more stable configuration, with a larger number of peptide linkages, increasing the susceptibility to enzyme hydrolysis (Deshpande & Damodaram, 1980; Carbonaro et al., 1992; Yokomizo & Vello, 2003).

Due to the great demand for soybean by the national food industry and export, Brazil has become the second largest soybean producer in the world (Santos et al., 2006). Thus, genetic improvement programs have emerged through biotechnology for the creation of genetically modified cultivars, which provide higher yield levels and reduced losses, increasing the productivity. *RoundUp Ready* (*RR*), for instance, was developed to be resistant to the herbicide called *Roudup*®. Once the *5-enolpyruvylshikimate-3-phosphate synthase* (CP4 EPSPS) protein, which acts in the shikimate pathway, is inserted, aromatic amino acids, which are essential to plant growth, are produced (Bollman et al., 2004).

An opposite view of genetically modified foods is the organic agriculture. It uses natural resources in order to keep the integrity and to maintain the productivity. In Brazil, the opening of the market for the organic products is recent. This movement began with support from the media and acceptance by the population. Today, there is a large niche for these products since there is great rejection by the population in relation to acquiring and consuming certain conventional products due to the use of agrotoxins, as well as to consuming the products derived from some type of new biotechnology (Borguini et al., 2003).

The objective of this study was to evaluate the cumulative effect of genetically modified and organic soybean (*Glycine Max L Merril*) consumption in the growth and development of the two generations of Wistar rats.

MATERIAL AND METHODS

Animal and diets

This study was approved by the Ethics Committee of the Antônio Pedro University Hospital, Federal Fluminense University (protocol # 57/02) and followed the norms of the Brazilian College of Animal Experimentation.

In this study sixty-four recently weaned, male, Wistar, *Rattus norvergicus* from the Experimental Nutrition Laboratory (LabNE) Department of Nutrition and Dietetic, School of Nutrition at the Federal Fluminense University-Niterói, RJ, were used. The test was divided into two phases and the first one was denominated generation F_0. In this phase, specific groups were formed and fed with organic soybean, genetically modified soybean and casein. When they reached fertility (90 days), these animals mated, giving place to the second phase called generation F_1, in which the same groups were formed as in generation F_0.

Experimental groups

The experimental groups received the following denominations: Organic Soybean Group, which received organic soybean based chow (GOF_0 and GOF_1); Genetically Modified Soybean Group, which received genetically modified soybean based chow (GGF_0 and GGF_1); Casein Group, which received casein based chow (GCF_0 and GCF_1).

These groups received their specific chow throughout their lives in both the generations (F_0 and F_1). The aproteic group (protein free) was also formed in each generation (GAF_0 and GAF_1), using the protein-free and isocaloric chow. All the animals received water and *ad libitum* chow and were kept confined in individual polypropylene cages in an environment with a constant temperature (24ºC ± 2ºC) and adequate illumination (light and dark cycles of 12 in 12 h).

Biological determination of protein

The Net Protein Ratio (NPR) was determined on the 14th day of the experiment, taking the weight gain of the test group plus the weight loss of GA in relation to test group protein consumption. At the end of 28 days for each generation, the Protein Efficacy Ratio (PER) was determined, taking the

weight gain of the test group in relation to test group protein consumption. The Coefficient of Alimentary Effectiveness (CAE) was determined by taking the weight gain of the test group, after 28 days, in relation to the test group chow consumption, according to Angelis (1995).
To prepare the different kinds of chow, both the varieties of soybeans underwent thermal treatment, according to Soares et al. (2005), aiming to reduce the antinutritional factors. Chow composition (Table 1) was based on *American Institute of Nutrition* (AIN) recommendations (Reeves et al., 1993), according to which, all diets had on average 10% protein (1.75% nitrogen) and 363.95 Kcal/100g.

Table 1 - Composition of the diets used for the assay (g/100g).

Food (g/100g)	***Casein***[1] **(GCF_0 and GCF_1)**	***Organic soy***[2] **(GOF_0 and GOF_1)**	**Transgenic soy**[3] **(GGF_0 and GGF_1)**	**Aproteic (GA)**
Protein	11.54	20.96	20.28	0.00
Starch[4]	61.41	57.23	58.04	72.95
Refined sugar[5]	10.00	10.00	10.00	10.00
Mineral mix[6]	3.50	3.50	3.50	3.5
Vitamin mix[7]	1.00	1.00	1.00	1.00
Soy oil[8]	7.00	2.81	2.94	7.00
Cellulose[9]	5.00	3.95	3.99	5.00
Choline bitartrate[10]	0.25	0.25	0.25	0.25
Cysteine[10]	0.30	0.30	0.00	0.00
Total	100.00	100.00	100.00	100

[1]Caseína-Comercial Rhosther Industria e Cormécio LTDA. [2]Jasmine Alimentos LTDA, [3]Bunge Alimentos.,[4]Maisena, [5]União, [6]Preparada segundo a AIN-93 (Rhosther), [7]Preparada segundo a AIN-93 (Rhosther), [8]Liza®, [9]Macrocel®, Blanver LTDA, [10]Rhosther

Chemical Composition Methods
The protein, lipid, ash and carbohydrate contents were determined after preparing the chow. This was carried out according to the *Association of Official Analytical Chemists* (AOAC, 1984) methods.

Statistical Analysis
The One Way ANOVA (Analysis of Variance) was applied to the results for the multiple analyses of variables at the $p \leq 0.05$ level. When statistical significance was detected, the Scheffe two-average test was applied, using the Bonferroni Coefficient, describing them in averages.

RESULTS AND DISCUSSION

Table 2 shows the composition of the different kinds of chow prepared for the test in hundredths. It was observed that the protein content varied from 10.95% to 12.96%, carbohydrates from 73.66 to 77.87%, lipids from 7.88 to 8.53%, moisture from 2.17 to 2.72% and ashes from 1.67 to 2.14%. The values shown in this table characterized the kinds of chow with equal composition in the hundredths.

Table 02 - Chemical composition of diets (g/100g).

Rations	**Moisture (%)**	**Lipids (%)**	**Ashes (%)**	**Protein (%)**	**Carbohydrates (%)**
GCF_0 and GCF_1	2.61 ± 0.51	7.88 ± 0.12	1.67 ± 0.00	10.95 ± 0.43	77.87
GOF_0 and GOF_1	2.17 ± 0.01	8.17 ± 0.11	2.14 ± 0.06	13.05 ± 0.31	74.45
GGF_0 and GGF_1	2.72 ± 0.06	8.53 ± 0.14	2.11 ± 0.08	12.96 ±1.18	73.66

Results are present as mean ± standard. GC – chow of control group; GO – chow of organic soy group; GG – kinds of chow of transgenic soy group.

When evaluating chow intake (Table 3), a similarity was observed in the same groups in different generations, in which the GGF_0 and GGF_1 had a lower ($p \leq 0.003$) value in comparison to the GOF_0 and GOF_1 in both the generations, and this was significantly lower ($p \leq 0.0001$) than the GCF_0

and GCF_1. Since these kinds of chow are isocaloric and isoproteic, the protein intake is a reflection of the chow intake. Therefore, protein consumption was proportional to chow consumption in all the groups for both the generations.

Sarwar et al. (1989) and Sgarbieri (1996) suported that the lipooxygenase enzyme (LOX) present in the soybean quickly oxidized the polyunsaturated fatty acids generating fatty acid peroxides, which was broken into aldehydes and volatile cetones. This caused the formation of the *beany flavor* and the consequent alteration in chow palatability. It could thus, be suggested that the lower consumption of the soybean based groups in relation to the control group could be related to the alteration in flavor caused by the enzyme action.

Knowing that there is a variation in total body protein caused by differences in diet protein quality, it is important to observe the variation in body weight as an overall reflection of ingested protein action (Angelis, 1995). In this study from one generation to the next, the variation in weight was not enough to identify a significant difference within the same group in different generations (Table 3). The GGF_0 and GGF_1 had values which were similar to the ones of the GOF_0 and GOF_1 and both were less than ($p<0,0005$) the GCF_0 and GCF_1 ones.

When were compare protein intake and the variation in body weight, it was observed that the GGF_0 and GGF_1 consumed a higher amount of protein compared to the GOF_0 and GOF_1 in both the generations, although it had a smaller weight variation in both the generations (Table 3). Sarwar et al (1989), found that cystine supplementation in balanced diets for the animals promoted better growth due to the improved incorporation of ingested protein.

Table 3 - Ration and protein intake, weight gain, protein intake (g)/weight (g) ratio and calorie intake (Kcal)/ weight (g) ratio.

		Ration intake(g)	Protein intake (g)	Weigth Gain (g)	Protein intake (g) / g weight	Calorie Intake (Kcal)/g weigth
Generation F_0	GCF_0	480.81 ± 10.87^{a}	49.44 ± 1.12^{a}	185.05 ± 3.06^{a}	0.26 ± 0.01^{a}	9.47 ± 1.56^{a}
	GOF_0	321.83 ± 7.79^{b}	33.06 ± 1.15^{b}	103.25 ± 2.56^{bc}	0.32 ± 0.02^{b}	11.37 ± 2.23^{b}
	GGF_0	407.45 ± 14.78^{c}	41.92 ± 1.16^{c}	89.65 ± 3.58^{c}	0.47 ± 0.03^{c}	16.69 ± 3.78^{c}
Generation F_1	GCF_1	445.35 ± 14.78^{a}	45.83 ± 0.75^{a}	176.56 ± 3.75^{a}	0.26 ± 0.01^{a}	9.22 ± 4.02^{a}
	GOF_1	331.15 ± 13.26^{b}	34.09 ± 1.49^{b}	105.78 ± 4.01^{bc}	0.32 ± 0.01^{b}	11.50 ± 3.51^{b}
	GGF_1	402.95 ± 13.22^{c}	41.40 ± 1.36^{c}	100.02 ± 4.89^{c}	0.41 ± 0.02^{c}	14.67 ± 2.06^{c}

Results are present as mean ± standard. Numbers followed by diferent superscript letter are statistically significant ($p \leq 0,05$, ANOVA followed by Scheffé and Boferroni tests).

According to Leung & Rogers (1975) and Miller & Payne (1964), both the lack and the overabundance of one or more amino acids implled a dynamic protein disequilibrium resulting in growth delays and organic alterations. Krajcovicova & Dibak (1980) suported that in situations where there was amino acid disequilibrium in the diets, the organism will have to metabolized the endogenous proteins to obtain the limiting amino acids, with accentuated wasting on the organism.

It is important to emphasize that GGF_0 and GGF_1 did not receive the L-cistine supplement, so there would be no interference in protein use for the chow based on this variety of soybean, since it is a genetically modified organism. When working with this same soybean, Soares et al. (2005) did not find differences in weight variation for the rats which were up to 291 days old. In the present study, it was observed that when dealing with the growth and development period, supplements become essential.

The Kcal/g body weight ratio (Table 3) was higher ($p<0.004$) for GGF_0 and GGF_1 in comparison to GOF_0 and GOF_1, respectively. The control group maintained a lower ratio ($p<0.01$) than the experimental groups, regardless of the generation. Thus, the genetically modified soybean based group revealed a consumption of 45 and 40% in generations F_0 and F_1, respectively, compared to the groups that consumed the organic soybean.

Carrying out biotests is important because they show essentially the amount of limiting amino acids that can be used by the animal, that is, the bioavailability of food protein amino acids (McLaugaulan & Keith, 1975).

The values found for PER compared to casein (Table 4) varied in generation F_0 from 84.43 to 56.84% for GOF_0 and GGF_0 respectively, and in generation F_1 from 81.20 for GOF_1 and 63.19% for GGF_1. It showed the superiority ($p<0.006$) of GOF_0 and GOF_1 compared to GGF_0 and GGF_1. Bressani (1995) found PER values equal to 2.44 for the isolated soybean and a PER of 2.23 for soy flour, from which the grain was thermally treated. Monteiro et al. (2004) found an average PER of 1.3 when they studied the genetically modified soybean linkages without the Kuntz Trypsin inhibitor and the LOX enzyme.

Freidman (1996) reported that a PER value of over 2.00 was generally related to good quality protein and a PER value below 1.50 was usually related to poor quality protein. Although the PER values were higher than the above referenced values, the experimental groups still showed poorer results than the GC, in spite of the generation.

Table 4 - PER, NPR and CAE of F_0 and F_1 generations.

	Generation F_0			Generation F_1		
	GCF_0	GOF_0	GGF_0	GCF_1	GOF_1	GGF_1
PER*	4.24 ± 0.15^a	3.58 ± 0.13^b	2.41 ± 0.22^c	3.83 ± 0.12^a	3.11 ± 0.17^b	2.42 ± 0.02^c
RPER (%)**	100.00	84.43	56.84	100.00	81.20	63.19
NPR*	5.49 ± 0.02^a	4.71 ± 0.15^b	3.67 ± 0.21^c	4.71 ± 0.15^a	3.81 ± 0.25^b	3.03 ± 0.13^c
RNPR (%)**	100.00	85.79	66.85	100.00	80.89	64.33
CAE*	0.38 ± 0.01^a	0.32 ± 0.03^b	0.22 ± 0.03^c	0.39 ± 0.04^a	0.31 ± 0.06^b	0.24 ± 0.01^c
RCAE(%)**	100.00	84.21	57.89	100.00	79.49	61.54

*Results are present as mean ± standard. Numbers followed by diferent superscript letter re statistically significant ($p \leq 0,05$, ANOVA followed by Scheffé and Boferroni tests). **RPER = relative PER; **RNPR = relative NPR ; **RCAE = relative CAE; GC = Control goup; GO = Organic soy group; GG = transgenic soy group.

The NPR has the capacity of showing that the protein influenced the animal growth and maintenance (Angelis, 1995). The NPR values for the groups with soybean decreased from one generation to the next (Table 4). This reduction was more evident when the NPR was analysed compared to casein, where although the groups showed lower values in generation F_0, these were not sufficiently different for statistically significant differences. A lower yield for the GGF_1 was observed in comparison to the other groups. However, the GCF_0 revealed a higher NPR than in all other groups. Gomes et al. (2000) found an NPR value of 3.70 for soybean, whereas Sarwar et al (1985) found a value of 3.53.

These values were similar to those found in the present study from the soybean based groups in generation F_1, from which the results were lower when compared to generation F_0.

The NPR values for soy flour based diets compared to casein were significantly different ($p<0.0004$), demonstrating that the quality of soy flour protein used as a protein source was not as effective as casein in order to promote the growth and maintenance of rats. It was observed that the thermal treatment used in soybeans was satisfactory since the values of biological indicators described in the literature, in which the beans were thermally treated, matched the values found in the present experiment.

The CAE values were similar for the GOF_0 and the GOF_1 in both the generations studied, presenting CAE values compared to casein of 84.21% in generation F_0 and 79.49% in generation F_1. The same was found for the GGF_0 with values of 57.89% for this group in generation F_0 and 61.54% in generation F_1, but with lower values ($p<0.0002$) for this group compared to the GOF_1 (Table 4). Since it is an alternative source of protein, a soybean, like other legumes, has been broadly studied. Analyzing several types of the beans, Rosa et al. (1998) found similar CAE values between the legume and casein, a result which was not found in this experiment, since in both the phases of the test, the soybean was statistically inferior to casein.

In a study, when evaluating the influence of a vegetal protein based diet and ratifying the importance of protein quality and quantity in the diet, Nepomuceno et al. (2000) observed that after three generations of rats, the animals that were fed this diet had lower body weight and low fertility rates.

Based on these results, it coud be concluded that the use of soybeans as a protein source for animals in growth stages must be done cautiously, because even when showing normal values, it would be important to draw attention to the legume's limitation in sulfurated amino acids. It was confirmed that even with L-cystine supplements, organic soybean was unable to reach similar levels as the standard protein based group concerning the biological indicators.

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RESUMO

Este estudo objetivou avaliar os efeitos cumulativos da utilização das sojas geneticamente modificada e orgânica em duas gerações de ratos. Utilizaram-se 64 ratos Wistar, de duas gerações consecutivas denominadas F_0 e F_1. Os animais de cada geração foram divididos em três grupos (n=8) alimentados com ração à base de soja orgânica, soja geneticamente modificada e caseína. Determinou-se o Quociente de Eficácia Protéica, Razão Protéica Líquida e Quociente de Eficácia Alimentar. Aos resultados aplicou-se ANOVA. Foi encontrada, em ambas as gerações uma diferença significativa ($p<0,05$) entre os grupos experimentais e o grupo controle. A suplementação dos grupos GOF_0 e GOF_1 com L-cistina proporcionou um melhor aproveitamento protéico em relação ao GGF_0 e GGF_1. Mostrando que a soja manteve seu aproveitamento, no entanto sempre inferior à caseína. Deve-se utilizar as sojas geneticamente modificada e orgânica com cautela na fase de crescimento, pois mesmo suplementada com L-cistina estas se mantiveram inferior ao grupo à base de caseína.

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