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RESPOSTA BIOLÓGICA, SISTÊMICA E REPRODUTIVA DE RATOS WISTAR ALIMENTADOS COM SOJA GENETICAMENTE MODIFICADA RESISTENTE AO GLIFOSATO

Tese apresentada ao Programa de Pós-Graduação em Biotecnologia da Universidade Federal de Pelotas, como requisito parcial à obtenção do título de Doutor em Ciências (área do conhecimento: Biossegurança de Alimentos).

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RESUMO

VENZKE, Janaína Guimarães. **Respostas biológica, sistêmica e reprodutiva de ratos Wistar alimentados com soja geneticamente modificada resistente ao glifosato.** 2009. 88f. Tese (doutorado) – Programa de Pós-Graduação em Biotecnologia. Universidade Federal de Pelotas, Pelotas.

A espécie vegetal geneticamente modificada mais cultivada no Brasil é a soja [*Glycine max* (L.) *Merr.*] resistente ao herbicida sistêmico pós-emergente glifosato, com 12,3 milhões de hectares cultivados. Essa soja foi obtida pela introdução do gene correspondente à enzima 5-enolpiruvilchiquimato-3-fosfato sintase (EPSPS, E.C 2.5.1.19, CP4), enzima da via de chiquimato, resistente ao glifosato, mantendo ativa a via biossintética de aminoácidos aromáticos. A modificação genética fez com que parâmetros preconizados pela legislação brasileira fossem revistos e o limite máximo permitido de glifosato nos grãos que era de 0,2 mg/kg aumentasse para 10 mg/kg. Embora o Brasil cultive há mais de dez anos a soja resistente ao glifosato e seja o terceiro maior produtor mundial dessa leguminosa, com 50% da produção nacional com genótipos transgênicos, poucos estudos têm sido conduzidos no país com o intuito de avaliar, em ensaios biológicos, o impacto dessa tecnologia sobre o meio ambiente, a qualidade e a segurança do alimento. Sendo assim, o presente trabalho objetivou estudar a influência do consumo de grãos de soja geneticamente modificada resistente ao glifosato (GM_{RR}) isogênica à convencional e não isogênica, tratada com este herbicida, sobre a qualidade nutricional, possíveis efeitos à exposição crônica, bem como efeitos sobre a fertilidade e o desenvolvimento de ratos Wistar e a possibilidade deste, desregular o sistema endócrino. A qualidade nutricional foi avaliada através do valor protéico mediante índices biológicos, em 30 machos recém desmamados, distribuídos em cinco grupos, alimentados por 28 dias com ração contendo 10% de proteína de soja GM_{RR} não isogênica, soja GM_{RR} da isogênica, soja convencional, leite (caseína) ou por 10 dias com ração aproteica. O ganho de massa corporal e o consumo alimentar das dietas não diferiu entre os tratamentos. Esse mesmo comportamento foi observado no que concerne às variáveis de crescimento e índices de balanço nitrogenado. Na exposição crônica de 40 machos e 39 fêmeas distribuídos em 4 grupos que consumiram dietas contendo sojas GM_{RR} (isogênica e não isogênica), soja convencional e grupo padrão sem soja,

sinais de toxicidade sistêmica nos machos foram evidenciados na neutrofilia, linfopenia do grupo que consumiu soja convencional em relação ao grupo padrão, e na hiperplasia linfóide dos pulmões nos animais que consumiram soja GM_{RR} e convencional; sinais de toxicidade reprodutiva foram observados através do aumento da massa relativa dos testículos e epidídimos e redução da concentração espermática. Nas fêmeas, os efeitos no sistema reprodutivo foram evidenciados através dos índices de fertilidade, e desmame. Estes resultados indicam que a qualidade protéica da soja modificada geneticamente é preservada, porém há uma associação de fatores que desencadeiam em uma provável desregulação endócrina. Outros estudos são necessários para elucidar o mecanismo de interação que provoca efeitos de toxicidade em machos e fêmeas alimentados com soja convencional e GM_{RR} que apresentam níveis detectáveis de glifosato.

Palavras chave: soja transgênica, geneticamente modificada, glifosato, toxicidade, ratos, alimento seguro, qualidade protéica.

ABSTRACT

VENZKE, Janaína Guimarães. **Biological, systemic and reproductive evaluation in Wistar rats feed on genetically modified soybean resistant to the glyphosate.** 2009. 91f. Tese (doutorado) – Programa de Pós-Graduação em Biotecnologia. Universidade Federal de Pelotas, Pelotas.

The most cultivated genetically modified plant in Brazil is the soybean [*Glycine max* (L.) *Merr.]* resistant to the systemic herbicide glyphosate. There are 12.3 millions of cultivated hectares. This soybean was obtained through the introduction of the gene that codes for the 5-enolpyruyilshikimic-acid3-phosphate synthase enzyme, (EPSPS, E.C 2.5.1.19, CP4), of the shikimic pathway, resistant to glyphosate that keeps active the aromatic amino acids biosynthetic pathway. To the soybean genetical modification the parameters in law were reviewed and the maximum glyphosate limit permitted in beans that was 0.2 mg/kg was increased to 10 mg/kg to GM_{RR} soybean. Although Brazil is the 3rd biggest worldwide producer, cultivates the GM_{RR} soybean for more than ten years, and represents 50% of the overall production of soybean in Brazil, just a few studies have been conducted in the country to evaluate in biological assays, the impact of this technology on the food quality and safety. Thus, the present work seeks for to evaluate the influence of the genetic modification of the soybean on the nutritional quality, on the possible effects of chronical exposition, as well as the effects on the fertility and the development of Wistar rats and the on the endocrine system. The nutritional quality was evaluated through the protein value, in 30 male rats, just weaned, distributed in five groups, fed along 28 days with the following diets, 10% protein ration GM_{RR} soybean no isogenic, GM_{RR} soybean isogenic, conventional soybean, milk (casein) or for 10 days with a non-protein diet. The weight gain and the food intake of diets did not present statistical relevance. The same behavior was observed in the variables of growth and the nitrogen balance study. In the chronical exposition of 40 male and 39 female rats distributed in four groups that consumed genetically modified soybean no isogenic and isogenic, conventional soybean and the standard group without soybean it signs of systemic toxicity on males have been evident in the neutrophilia, lymphocytopenia in the group conventional soybean compared with the standard group, lymphoid hyperplasia of the lungs in the groups without soybean; the signs of reproductive toxicity through of

the increase on relative weight of the tests and epididymis and also through the decrease of the sperm concentration. On the females the effect on the reproductive system became evident through the fertility pregnancy and weaning index. These results point out that the protein quality of the genetically modified soybean is preserved, but there is an association of factors that trigger a probable endocrine disruption. Studies are necessary to elucidate the mechanism of interaction that cause toxicity on males and females fed with conventional soybean and genetically modified soybean that presented detectable glyphosate levels.

Keywords: genetically modified soybean, glyphosate, toxicity, rats, transgenic soybean, food safety, protein quality.

SUMÁRIO

INTRODUÇÃO

Com o crescimento da população no mundo, principalmente a partir da segunda guerra mundial, os programas de melhoramento de espécies produtoras de grãos buscaram selecionar cultivares com elevada produtividade. Com a evolução nas áreas do melhoramento assistido e em associação com técnicas de bioquímica, biologia molecular, cultura de tecidos e transformação genética, tornou-se possível a transferência dirigida de genes entre espécies e dentro da mesma espécie. Por essas estratégias pode-se identificar e, eventualmente remover, características indesejáveis responsáveis por reações alérgicas, toxicidade ou predisposição a doenças (COSTA; BORÉM, 2003).

Nos Estados Unidos, no ano de 1994, foi comercializado o primeiro alimento geneticamente modificado, o tomate Flav Savr®, da empresa Calgene, que apresenta maior durabilidade pós-colheita (ZANCAN, 1999). Porém, esse genótipo foi removido do mercado dois anos após o lançamento, devido à inferioridade agronômica em relação a outros híbridos mais produtivos e produtores de frutos com maior qualidade. Desde então, outras espécies modificadas geneticamente vêm sendo cultivadas em áreas crescentes em diversos países das Américas, Europa, África e Oceania. O país que mais aumentou o cultivo de plantas geneticamente modificadas em 2005 foi o Brasil, com um incremento de área estimado em 4,4 milhões de hectares (9,4 milhões de hectares em 2005 comparados aos 5 milhões em 2004), seguido pelos incrementos nos Estados Unidos (2,2 milhões de hectares), na Argentina (0,9 milhões de hectares) e na Índia (0,8 milhões de hectares). O maior aumento absoluto na área de lavouras biotecnológicas em 2006 foi nos Estados Unidos (4,8 milhões de hectares), seguido pela Índia (2,5 milhões de hectares) e Brasil (2,1 milhões de hectares). A superfície cultivada com variedades transgênicas em 2008 atingiu 125 milhões de hectares, envolvendo mais de 25 países e dezenas de espécies (ISAAA, 2009). O Brasil, com 15,8 milhões de hectares cultivados com soja, algodão e milho, está na terceira posição dos maiores produtores de transgênicos. Estados Unidos com 62,5 milhões de hectares e Argentina com 21 milhões de hectares lideram a produção de transgênicos no mundo. Nestes dois

países, a área com geneticamente modificados representa 80% do total cultivado (ISAAA, 2009).

No cenário mundial o Brasil, com produção superior a 50 milhões de toneladas, para a qual são cultivados mais de 20 milhões de ha/ano, tem se destacado como um dos principais produtores de soja do mundo (AGROLINK, 2008). A produção da soja, em sua maioria, é destinada ao processamento para a produção de óleo, e responde pela maior percentagem dos grãos exportados. Assim, a soja destaca-se como uma das comodites agrícolas de maior importância na pauta de exportação, produzindo importante impacto no quadro econômico da agricultura.

As plantas concorrentes constituem um dos principais fatores limitantes para a produção agrícola dessa cultura. Tipicamente, estas plantas são controladas com uma combinação de práticas culturais (plantio direto, aração e gradagem) e métodos químicos, como o uso de herbicidas. A soja transgênica *Roundup Ready* (RR), pela modificação genética da enzima EPSPS (5-enolpiruvilshiquimato-3-fosfato sintase), tornou-se tolerante ao herbicida glifosato. Esse herbicida é um eficiente inibidor da enzima EPSPS, o que provoca o bloqueio da síntese de aminoácidos aromáticos nas plantas. Com a presença do glifosato na planta, e restrição da produção dos aminoácidos aromáticos, há também bloqueio da síntese de proteínas, levando à morte celular. Na soja transgênica, na qual foi inserido um alelo do gene que codifica a enzima 5-enolpiruvilshiquimato-3-fosfato sintase, que possui baixa afinidade pelo glifosato, a rota metabólica dos aminoácidos aromáticos não é inibida, conferindo assim, tolerância ao herbicida glifosato (HARRISON et al., 1996). O gene inserido nesses genótipos de soja foi obtido da bactéria *Agrobacterium tumefaciens*, estirpe CP4 (COSTA; BORÉM, 2003).

Os agrotóxicos, de modo geral, são extensivamente utilizados no controle de pragas, doenças e plantas concorrentes de diversas culturas, como milho, arroz, feijão, café e, especialmente, soja, que, segundo o Sindicato da Indústria de Defensivos Agrícolas (SINDAG, 2002), é a cultura que mais consome agrotóxico, requerendo, portanto, especial atenção da comunidade científica. Dentre os agrotóxicos mais usados na cultura de soja destaca-se o glifosato, herbicida sistêmico pós-emergente, de fundamental importância após a criação e a introdução

do cultivo da soja transgênica *Roundup Ready* (RR) (HOEF et al., 1998). Doravante, essa soja será denominada de geneticamente modificada (GM_{RR}).

A modificação genética na soja que conferiu resistência ao glifosato, fez com que parâmetros preconizados pela legislação fossem revistos e, o limite máximo permitido de glifosato nos grãos que era de 0,2 mg/kg para a soja convencional aumentou para 10 mg/kg para a soja geneticamente modificada (ANVISA, 2008). A possibilidade de manifestação de distúrbios de fertilidade decorrentes da exposição ao glifosato tem recebido especial atenção devido às repercussões sobre o ser humano, exposto via alimentação e/ou ocupacionalmente (EPA, 1996a). Atualmente, parte significativa dos xenobióticos (substâncias estranhas ao organismo) presentes no ambiente são resíduos de agrotóxicos utilizados nas lavouras ou produtos de degradação dessas moléculas (BOHN et al, 2008). Essas substâncias, persistentes no ambiente e/ou bioacumuladas nos organismos, têm sido apontadas como capazes de alterar o sistema endócrino, e, portanto, denominadas de desreguladores endócrinos (STEVENS et al., 1997). Nesse contexto, esse projeto propôs um estudo da segurança do alimento transformado com *epsps-cp4* já presentes em cultivares de soja geneticamente modificada resistente ao glifosato.

Segurança de alimentos geneticamente modificado

A avaliação da segurança de alimentos deve nortear pelo princípio da precaução, a fim de prever e preparar a liberação de organismos geneticamente modificados e seus produtos na cadeia alimentar e seus impactos na saúde e no ambiente. As normas de segurança do uso de alimentos geneticamente modificados no Brasil são baseadas na Instrução Normativa nº 20 da Comissão Técnica Nacional de Biossegurança (CTNBio), de 11.12.2001, onde questões relativas às características e potencial de alergenicidade ou de toxicidade da planta receptora e do organismo doador devem ser avaliados. A FAO/OMS descreve através do *Codex Alimentarius*, métodos de avaliação da inocuidade em alimentos derivados de plantas e microrganismos geneticamente modificados (CAC, 2003), recomendando uma avaliação prévia à comercialização de produtos geneticamente modificados, caso a caso, incluindo os efeitos diretos (do gene inserido) e os não desejados em consequência da inserção do gene. Efeitos de toxicidade, alergenicidade, componentes com propriedades nutricionais tóxicas e estabilidade do gene, são

algumas das avaliações da inocuidade dos alimentos geneticamente modificados propostas pela FAO/OMS (WHO, 2005).

As plantas transgênicas aprovadas para o cultivo comercial nos Estados Unidos, tiveram sua liberação baseada no princípio da equivalência substancial. Para a determinação da equivalência substancial, a composição do produto geneticamente modificado deve ser comparada à composição do análogo convencional, cultivada sob condições ambientais similares, uma vez que estas podem levar a diferenças na composição não relacionados à modificação genética (WATANABE; NUTTY,2002). Esse conceito tem sido alvo de críticas devido à falta de critérios mais rigorosos do ponto de vista do consumidor e da saúde pública (MILLSTONE et al, 1999), e, a rigor, genomicamente, elas não são equivalentes nem iguais (NODARI; GUERRA,2003). Esse princípio deveria ser abandonado em favor do princípio da precaução que inclui testes biológicos, toxicológicos e imunológicos que garantam a segurança do produto (NODARI; GUERRA, 2001). No Brasil, a legalização do cultivo da soja GM_{RR} ocorreu baseada em bases científicas, porém majoritariamente obtidas em condições edafoclimáticas distintas daquelas das regiões de produção. Esse fato é importante, tendo em vista que foi encontrado que essas variáveis afetam o comportamento de soja GM_{RR} (BOHN et al., 2008).

A introdução de novas tecnologias sempre foi acompanhada de controvérsias. O princípio da precaução considera um alimento seguro quando existe uma certeza razoável de que nenhum prejuízo resultará de seu consumo sob as condições de uso estipuladas. Para isso se faz necessário aplicar testes para a avaliação da toxicidade de substâncias específicas caso a caso. Isso não ocorreu quando da autorização de cultivo da soja GM_{RR} no Brasil.

O requerimento mínimo necessário para demonstrar a segurança do consumo do alimento em longo prazo é a realização de um estudo subcrônico de 90 dias. Estudos mais prolongados podem ser necessários se os resultados indicarem efeitos adversos (KUIPER et al, 2001).

Os protocolos para a verificação da toxicidade de alimentos produzidos por biotecnologia não diferem do que é recomendado para analisar a segurança de qualquer outro alimento (COSTA; BORÉM, 2003). Uma das considerações na avaliação da segurança desses alimentos é o possível reflexo da modificação genética em seus níveis de antinutrientes e toxinas.

Ensaios para avaliação da qualidade protéica

Quantitativamente, os principais constituintes da soja são a proteína e o óleo, sendo que o grão de soja contém cerca de 40% de proteína e 20% de óleo, considerando-se a massa seca, é uma boa fonte de minerais e vitaminas do complexo B, mas pobre em cálcio. A soja contém, ainda, componentes conhecidos como fatores antinutricionais, que incluem inibidores de proteases, lectinas, oligossacarídeos, fitatos e saponinas (PANIZZI; MANDARINO, 1994). Produtos de soja requerem tratamento térmico para melhorar seu valor nutritivo, o que é alcançado, em parte, pela inativação dos inibidores de proteases.

A avaliação da qualidade protéica se dá através de ensaios biológicos, durante 14 dias ou 28 dias, com ratos Wistar, recém desmamados, conforme as recomendações da AOAC (1996). Nitrogênio da dieta, nitrogênio das fezes, nitrogênio da urina são determinados e relacionados ao consumo alimentar e ganho de massa corporal. Com esses dados, determina-se Digestibilidade Verdadeira, Coeficiente de Eficiência Protéica (PER), Razão Protéica líquida (NPR) e Utilização Protéica líquida (NPU).

A instrução normativa nº 20 da CTNBIO de 11.12.2001, dispõe de normas para avaliar a segurança do alimento de plantas geneticamente modificadas ou de suas partes. Dentre as normas relativas à qualidade nutricional da proteína, está à necessidade de se avaliar a alteração na estrutura, composição ou teor dos nutrientes, modificando ou não, a qualidade nutricional, ou afetando a digestibilidade. Esta normativa foi elaborada após a introdução e cultivo da soja GM_{RR} no país.

Ensaios de toxicidade sistêmica

Poucas variedades tradicionais de alimentos têm sido objeto de avaliações toxicológicas como os produtos oriundos da biotecnologia, uma vez que, os produtos tradicionais por causa de seu consumo comum são reconhecidos como seguros. O principio da precaução considera um alimento seguro quando existe certeza de que nenhum prejuízo resultará do seu consumo sob as condições de uso (KUIPER et al., 2001). Portanto, se fazem necessários testes para avaliação da toxicidade de substâncias específicas caso a caso.

Os requerimentos mínimos necessário para demonstrar a segurança do consumo do alimento em longo prazo é a realização de um estudo subcrônico de 90 dias. Estudos mais longos podem ser necessários se os resultados do estudo subcrônico indicar efeitos adversos (KUIPER et al., 2001).

Os protocolos para a verificação da toxicidade de alimentos produzidos por biotecnologia não diferem do que é recomendado para analisar a segurança de qualquer produto (COSTA; BORÉM, 2003). Inicialmente, caracteriza-se a relação dose-resposta e, assim, os diversos níveis de efeito nos parâmetros tais como ganho de massa relativa, consumo relativo de ração, variáveis hematológicas, bioquímicas, urinálise, massa relativa de órgãos e análise histopatológica. Uma das considerações na avaliação da segurança desses alimentos é o possível reflexo da modificação genética em seus níveis de antinutrientes e toxinas (GMSO, 2008).

Ensaios em toxicologia reprodutiva

O sistema reprodutivo, tanto masculino como feminino, depende da interação de vários hormônios, que são suscetíveis à interferência por diversos xenobióticos. A exposição a essas substâncias estranhas pode ocorrer em distintas fases de desenvolvimento e, segundo estas, pode provocar seus efeitos de forma mais intensa e/ou irreversível. A exposição aguda ou crônica de animais adultos leva normalmente à falhas na reprodução. Entretanto, quando a ação desses agentes é direta sobre o aparelho reprodutor maduro, pode permitir a reversibilidade do distúrbio de fertilidade ao eliminar-se a fonte de exposição. Os efeitos gerados pela exposição pré e perinatal levam frequentemente a distúrbios irreversíveis, pois as alterações são produzidas durante a formação e/ou diferenciação dos órgão sexuais. Também é importante ressaltar que o período transcorrido entre a exposição nessa fase de vida e a manifestação dos sintomas geralmente é longo, e somente nas fases peripubertal e adulta surgirão os efeitos sobre o sistema reprodutivo. Estudos na área de toxicologia reprodutiva priorizam a avaliação dos efeitos das substâncias químicas sobre o sistema hormonal de animais e do homem, utilizando ratos como modelo experimental, devido à facilidade na condução dos ensaios *in vivo* (NEUBERT et al., 1977).

Estudos epidemiológicos postulam que, nos últimos 50 anos, o número de espermatozóides e a qualidade destes vêm sendo reduzidos no homem

(BENDVOLD et al., 1991; AUGER et al., 1995). A hipótese que vêm sendo investigada é a de que substâncias contaminantes ambientais e alimentares possam ter influência sobre o sistema endócrino (SHARPE et al., 1993).

Alterações específicas, como anomalias congênitas, podem ocorrer espontaneamente (mutações causadas por fatores endógenos) ou pela influência de substâncias tóxicas (efeitos teratogênicos). Entretanto, a incidência de anomalias espontâneas é prevista dentro das espécies de animais modelo, como também para a espécie humana (EPA, 1996b).

Diversos fatores, como tempo, espaço, custo e, principalmente, fatores éticos, limitam estudos toxicológicos sobre a reprodução humana e animal, tornando imprescindível o uso de modelos animais para avaliação de possíveis efeitos da exposição a determinados agentes químicos sobre a função reprodutiva (AMANN, 1982; HAYES, 1994).

A avaliação toxicológica dos prováveis efeitos dos desreguladores endócrinos sobre os sistemas hormonais humano e animal segue protocolos padrões para testes de toxicidade reprodutiva conforme guias estabelecidos por *Food and Drug Administration* (Administração de Drogas e Alimentos – FDA). Incluindo testes de fertilidade e performance reprodutiva de machos e fêmeas, toxicologia do desenvolvimento e teratologia e toxicidade peri e pós-natal. Adicionalmente, testes multi-gerações são preconizados pela *Environmental Protection Agency* (Agência de Proteção Ambiental – EPA) (THOMAS, 1995; EPA, 1996a). Para a realização desses testes, utilizam-se, dentre outros, ratos e coelhos como modelos experimentais, devido ao porte, prolificidade, informações disponíveis sobre a fisiologia das espécies e também custo de manutenção dos ensaios *in vivo* (AMANN, 1982; NEUBERT et al., 1977). Atualmente, a *Organization for Economic Co-operation and Development* (OECD), regulamenta os protocolos de toxicidade aceitos internacionalmente para aprovação de produtos.

Fases de desenvolvimento fisiológico do modelo biológico usado

Convencionalmente utiliza-se a espécie *Ratus norvergicus* e, frequentemente, a linhagem Wistar para avaliação da qualidade protéica, efeitos sistêmicos e efeitos sobre reprodução e fertilidade. As diferentes fases de desenvolvimento pós-natal dos ratos segundo Ojeda e Urbanski (1998) são:

I. Neonatal: primeira semana de vida;
II. Infantil: do 8º dia até o 21º dia (quando se efetua o desmame); a partir o 15º dia o rato continua a amamentação e inicia a introdução da ração.
III. Crescimento: o pico de crescimento infantil é do 21º (recém desmamados) até o 28º dia;
IV. Juvenil: do 30º ao 32º dia nas fêmeas e 35º dia nos machos;
V. Peripuberal: período de duração variável que, nas fêmeas está em torno do 38º dia e, nos machos, entre o 55º e o 60º dia.

Os machos atingem a maturidade sexual entre 60 e 75 dias de vida. Sua fertilidade máxima situa-se entre 100 e 300 dias, e a senescência reprodutiva se dá ao redor dos 360 dias de idade. Nas fêmeas, a maturidade sexual é atingida entre 60 e 75 dias de vida, e sua fertilidade máxima encontra-se entre 90 e 120 dias e a senescência reprodutiva se dá ao redor dos 360 dias de idade. As ratas são animais poliéstricas anuais, ou seja, manifestam vários ciclos estrais de quatro a cinco dias, ao longo do ano (COBEA,1996). Estro de 10 a 20 horas, com ovulação espontânea. O período de gestação tem duração de 20 a 22 dias, e o tamanho da ninhada varia entre 5 a 15 filhotes (COBEA,1996; CHAHOUD; KWASIGROCH, 1977).

Objetivos

I. Objetivo geral

Avaliar os efeitos do consumo da soja geneticamente modificada, sobre a biodisponibilidade protéica e o sistema reprodutivo de ratos Wistar.

II. Objetivos específicos

- Avaliar os efeitos do consumo da soja geneticamente modificada sobre variáveis biológicas de machos recém desmamados, que determina a digestibilidade e a biodisponiblidade da proteína.
- Avaliar os efeitos do consumo da soja geneticamente modificada sobre variáveis de toxicidade sistêmica em machos expostos cronicamente, incluindo variáveis clínicas, hematológicas, bioquímicas e histopatológicas.

- Avaliar os efeitos do consumo da soja geneticamente modificada sobre variáveis de toxicidade reprodutiva de machos e fêmeas da geração paterna e da segunda geração exposta pré e perinatalmente.

ARTIGO 1

Artigo sobre a avaliação da qualidade protéica da soja geneticamente modificada.

Biological evaluation of genetically modified soybean in Wistar rats

Artigo formatado segundo as normas da revista *Food Chemistry*.

Biological evaluation of genetically modified soybean in Wistar rats

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Abstract

The present study aimed to evaluate the conventional soybean protein quality and compare it to the isogenic genetically modified soybean (GM_{RR}) of a known source and to the one used no isogenic by producers. The protein quality was evaluated through biological indexes. Young, male Wistar rats (N = 30), were alocated into five groups, and feed either with a 10% protein diet (GM_{RR} no isogenic and GM_{RR} BRS 245 isogenic, the conventional soybean BRS 137, casein) during 28 days, or with a non-protein diet during 10 days. The weight gain and the feed consumption of diets were not affected by genetic modification. The same behavior was observed in terms of growth, as well as, the indexes of the nitrogen balance study the three soybean groups did not present statistical difference among them. Thus, we concludes that the protein quality of the GM_{RR} soybean is equivalent to the conventional soybean.
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Keywords: Protein quality, food quality, soybean protein, GM_{RR}, biological evaluation, Wistar rats.

1. Introduction

The soybean is an agricultural product that is of special concern to the whole world because it represents 70% of the worldwide consumption of proteins (James, 2007). Due to its application versatility in products to animal and human nourishment it assumes an important economical role in the national and international markets.

In the worldwide scene, the soybean production will probably have an increase of 6.2% in the 2008/09 crop. Brazil, the second biggest worldwide producer of soybean will participate with 59 millions of tons for which more than 20 millions ha/year are cultivated; it is something like 25% of the worldwide crop that is 234.7 millions of tons (Agrolink, 2008). The soybean production is mostly destinated to the oil production, and is responsible for the biggest percentage of exported grains. Thus, the soybean stands out in the national agricultural context, producing an important impact on the agricultural economical panorama (Embrapa, 2008).Data from the International Service for the Acquisition of Agri Biotech Application (ISAAA) (James, 2007) demonstrate that the area of culture increased 12% or 12.3 millions of hectares in 2007, totalizing 114.3 millions of hectares, involving more than 16 countries and dozen of important species in the production of supplies (James, 2007).

The Roundup Ready (RR) genetically modified soybean ($GM_{RR)}$, for the genetical modification of the enzyme EPSPS (5-enolpiruvilshiquimato-3-phosphate syntase), became tolerant to the glyphosate herbicide. This herbicide is an efficient inhibitor of the enzyme EPSPS, blocking the synthesis of the aromatic amino acids in plants. The presence of glyphosate in the plant and the restriction in the aromatic amino acids production also causes the blockage of the protein synthesis leading the cell death. On the other hand, the GM_{RR} soybean that codes for an enzyme EPSPS variant which has a low affinity with glyphosate, does not inhibit the aromatic amino acids metabolic pathway so, it gives tolerance to the glyphosate (Harrison et al., 1996). The gene inserted in this species was cloned from *Agrobacterium tumefaciens* CP4 (Costa e Borém, 2003).

The soybean nutritional properties, such as the high proportion of protein with the suitable quality, fibers, minerals or the reduced amount of saturated fat and the absence of cholesterol turn the soybean raw material highly attractive for the use in the feeding industry, mainly in the products based in cereals and meat (Amaya-

Guerra, alanis-Guzman, Saldivar, 2004; Vega and Felício, 1987). The soybean is also source of photochemical substances, such as flavonoyds (Bohn *et al*, 2008). Recent studies have demontrated the relation between the consumption of soybean and the reduction of non-infectious chronicle diseases, like cardiovascular diseases and some kinds of cancers and osteoporosis (Esteves and Monteiro, 2001; Lichtenstein, 1998; Morais and Silva, 2000). All this factors contribute to the increase of soybean in the feed industry. The soybean unpleasant and residual taste and smelling due to the presence of several organical composites are still a challenge to the technological research associated to the industry (Morais and Silva, 2000. Sarwar, 1997; Vasconcelos *et al*, 2001). The GM_{RR} soybean has been cultivated for more than ten years and it is estimated that it represents 50% of the soybean total production in Brazil (Embrapa, 2008). Just a few studies have been carried out concerning to the genetically modified soybean in relation to the nutritional quality. Some specific parameters like weight gain and feed consumption have been reported. In some cases, changes in the nutritional performance have been observed; it shows that intentional effects can occur in the genetical modification (EFSA, 2008).

Therefore, considering the huge application of soybean in the feed industry and the use of genetically modified soybean in the feed industry, the present study aimed to evaluate the protein quality of conventional soybean compared to the protein quality of the isogenic GM_{RR} soybean of a known source (EMBRAPA®) and the no isogenic GM_{RR} soybean obtained from a producer of Tupanciretã-RS-Brazil.

2. Materials and methods

2.1. Material

Genetically modified soybean (GMS_{RR}) BRS 245 event and the isogenic non-genetically modified soybean (NGMS) BRS 137 event were from Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA - Londrina, Paraná, Brazil) and no isogenic GM_{RR} soybean from the producer (PGM_{RR}) was from Tupanciretã (Rio Grande do Sul, Brazil) BRS charrua RR.

2.2. Sample preparation

Soybeans were submitted to heat treatment at 100 ºC for 5 min (autoclaving) for the possible adverse effects of protease inhibitors (trypsin and chymotrypsin) and lectins (Antunes & Sgarbieri, 1980). After that, the soybean was milled, homogenized and sieved through 60 meshes. The flour was stored at cold temperature (± 4 ºC) for subsequent chemical analysis and for preparation of the rations.

2.3. Animals test

Young, 21-25 days old, white male Wistar rats, weighing 53-63 g, were obtained from the Central Animal House, Federal University of Pelotas, Rio Grande do Sul, Brazil. The rats were randomly distributed into five groups, each of them consisting of ten rats. The animals were housed individually in stainless steel metabolic cages designed for separate collection of faeces and urine. Cages were located in a room with a 12 h light/dark cycle, at a temperature of 21 ± 2 ºC, fitted with an appropriate ventilation system.

2.4. Basic Chemical composition

Percentages of moisture, fat, protein, and ash were determined by the AOAC method (1996).

2.5. Composition of diets

Three test protein diets (GMS_{RR}, NGMS, and PGM_{RR}), one standard protein diet (casein) and one non-protein diet (basal), used as controls to estimate the endogenous nitrogen excretion of the rats, were prepared by mixing the protein source and others ingredients (Table 1). Standard and test protein diets were adjusted to 10% protein, whereas, non-protein diet was devoid of protein. In addition to the protein sources, the diets contained vitamin and mineral mix (AIN-93G) according to Reeves et al. (1993). For the preparation of the diets, ingredients were homogenized and passed through a 60-mesh sieve to ensure uniform distribution of

minerals and vitamins. All the diets were analyzed for their moisture, protein, lipid, and ash contents (AOAC, 1996).

2.6. Growth experiment

Protein efficiency ratio (PER) was determined according to AOAC (1996). Animals were initially weighed and food and water were given *ad libitum.* Rats fed on different experimental diets and control diets were weighed for four weeks and the gain in weight during this period was recorded. The consumed protein was calculated from the consumed nitrogen, based on diet's nitrogen content. Protein efficiency ratio (PER), food efficiency ratio (FER), and food transformation index (FTI) were calculated by following formulas, as described by FAO/WHO (1989).

PER = gain in body weight (g) / protein consumed (g)
FER = gain in body weight (g) / food consumed (g)
FTI = food consumed (g) / gain in body weight (g)

The corrected protein efficiency ratio (C-PER) was calculated according to Chapman et al. (1959), where 2.5 as standard value for casein:

C-PER = PER X 2.5 / Determined PER for reference casein

At the end of four weeks, final body weights of individual animal were recorded. After euthanasia, the liver, right and left kidneys, and spleen were carefully dissected and immediately weighed. The weights of these organs were expressed percentage of final body weight.

2.7. Nitrogen balance studies

Nitrogen balance studies were carried out during the experiment. During the second and third consecutive weeks, faeces and urine of each rat were collected separately. The faeces were oven-dried at 100 ºC for 24 h. The dried samples were ground to 20 meshes. The concentration of nitrogen in urine and faeces was estimated by microKjeldahl method according to AOAC (1996). The non-protein

dietary group was used for measurement of metabolic faecal nitrogen and endogenous urinary nitrogen. The data obtained from this experiment were used to calculate nitrogen absorved (NA), nitrogen retention (NR) apparent nitrogen digestibility (AND), true digestibility (TD), biological value (BV), net protein retention (NPR) and net protein utilization (NPU), as described by FAO/WHO (1989); and protein retention efficiency (PRE), as described by Bender and Doell (1957), by employing the following formulas:

$NA = NI - NF_1$

$NR = NI - (NF_1 + NU_1)$

$AND = NI - NF_1 / NI \times 100$

$TD = NI - (NF_1 - NF_2) / NI \times 100$

$BV = NI - (NF_1 - NF_2) - (NU_1 - NU_2) / NI - (NF_1 - NF_2) \times 100$

$NPU = BV \times TD / 100$

NPR = Weight gain of test group + Weight loss of protein – free group / weight of test protein consumed

$PRE = NPR \times 16$

where, NI is nitrogen intake of animals fed with the test diet; NF_1 the nitrogen excreted in faeces of animals fed test diet; NF_2 the nitrogen excreted in faeces of animals fed protein-free diet (basal diet); NU_1 the nitrogen excreted in urine of animals fed test diet; NU_2 the nitrogen excreted in urine of animals fed protein-free diet (basal diet).

2.8. Statistical analysis

All the parameters were calculated for each rat. Analysis of variance (ANOVA) was used to analyze the mean differences between the dietary treatments. The least

significant different (LSD) values were computed in case the F-test showed significant difference. A significant difference was considered at a level of $P < 0.05$.

3. Results and discussion

3.1 Proximate chemical composition

The table 2 shows the chemical composition of the casein diet (control diet), GM_{RR} soybean diet and non- GM_{RR} soybean diet. The chemical composition was similar for the four diets (casein, GMS_{RR}, NGMS and PGM_{RR}), because the diets were prepared according to AIN-93 (Reeves,1993) and adjusted to 10% protein, the minimum amount to assure that the animal model use protein just to the growth, avoiding other metabolic ways, to obtain energy for example (Bender and Doell,1957). The table 3 shows protein content of the GM_{RR} soybean no isogenic (33.54%), the GM_{RR} soybean (35.94%) and non- GM_{RR} isogenics (37.53%), are lower than the ones found in varieties of this leguminous plant cultivated in Brazil (41%), China and The United States (42%) (Grieshop and Faheyn Jr., 2001). Mascarenhas *et al* (1996) detected tenors similar to those and checked that the conditions of management of the soil and especially environmental conditions can affect the accumulation of proteins.

3.2 Growth experiment

The data on the growth studies of the rats treated with GM_{RR} soybean and no-GM_{RR} and casein are shown in table 4.

The figure 1 show the cumulative body weight gain of the rats fed on control and test diets. During the 28 days of treatment, a linear increase in body weight of rats was observed in all groups (figure 1). Rats fed on control diet (casein diet) grew faster than rats fed other diets, however, grew at a rate that was not significantly different ($P<0.05$) from casein diet (table 4).

Food intake after 28 days on the GMS_{RR}, NGMS, PGM_{RR} and casein (standard protein) diets were the highest, 349.46 g, 374.98 g, 386.79 g and 320.33 g, respectively, but these not differed significantly ($P>0.05$). Body weight gain on the control group (casein diet) was the highest (94.46g per rat) after 28 days, but the

difference was not significant (P>0.05) in comparison to the PGM_{RR}, NGMS and GMS_{RR} diets, 72.49, 85.85 and 78.42 g per rat, respectivelly.

Food Efficiency Ratio (FER) was the highest on the casein diet (0.30). This value differed significantly (P<0.05) from the PGM_{RR} diet (0.21), NGMS diet (0.23) and GMS_{RR} diet (0.20). The food transformation index (FIT) was better for rats fed on casein diet. This group required less feed to increase one gram (3.40) compared to those fed on PGM_{RR} (4.90), NGMS (4.39) and GMS_{RR} (5.01) diets. Statistical analysis showed a similar trend to the food efficiency ratio (table 4). The genetical modification in the soybean did not affect the FER and FIT to the rats.

Casein diet had a protein efficiency ratio (PER) of 2.67, this value was significantly higher (P<0.05), than PGM_{RR}, NGMS and GMS_{RR} diets (1.91, 2.19 and 1.75 respectively). The PER variations between these formulations (PGM_{RR}, NGMS and GMS_{RR}) were no significant (P>0.05). Protein quality, weight gain and PER are inter-related. The better the protein quality, the higher the weight gain and the higher would be the PER and vice versa (Sgarbieri, 1996).

The corrected protein efficiency ratio (C-PER) value of three test diet 1.79 (PGM_{RR}), 2.05 (NGMS), and 1.64 (GMS_{RR}) was significantly lower (P<0.05) than the casein diet (2.50). The difference between the PER and C-PER of soybeans diets was not significant (P>0.05).

In relation to the results presented in the biological study (table 4), the soybean protein promoted weight gain no significantly different from the casein diet (casein), but the PER, CPER, FET and FIT, values were statistically different (P<0.05), once the consumptions of rats ration and protein did not differentiate statistically among the groups of diets (P>0.05).

In table 4 it can be seen the similarity among the soybean diets concerning the rats' growth. According to these results, it can also be seen that the introduction of vegetal products reduce the biological use of rats food, because the protein sources of vegetal origin are less digestible than the animal ones (casein diet). In studies that used experimental conditions similar to the present work (Naves et al, 2004; Silva et al, 2006), the effectiveness of the feed conversion from a ration containing rice and beans (Naves et al, 2004) and another containing soybean bran and soybean (Silva et al, 2006) as sources of protein had their values similar to the ones found on this study (table 4).

However, our results of body weight gain, PER, FER and FTI, for the soybeans proteins show that the protein quality between the GM_{RR} soybean diet and the non- GM_{RR} soybean diet are not different.

3.3 Nitrogen balance experiments

The data on the nitrogen balance studies of genetically modified soybean are shown on table 5. The nitrogen consumed, nitrogen absorbed and nitrogen retained were similar for casein and the test group diets (PGM_{RR}, NGMS and GMS_{RR}). The nitrogen retained was positive in all groups, showing that nitrogen intake was larger than the faecal and urinary excretion of nitrogen.

The results indicate that the true digestibility (TD) was significantly lower (P<0.05) for soybean no isogenic diet (92.23%) than the TD value obtained for rats on casein diet (96.69%). The other diets (NGMS, GMS_{RR}) were similar (P>0.05) to the PGM_{RR} diet and casein diet. The apparent nitrogen digestibility (AND) was similar (P>0.05) among the soybean diets and they were significantly lower (P<0.05) for the rats fed on the casein diet. The statistical difference presented by the TD parameter in the genetically modified soybean diet from the producer in relation to the casein is possibly be associated with the vegetal tissue characteristics that limit the biological use in relation to the animal protein (casein) (Sarvar, 1997; Vega and Felício, 1987). It can be observed that the genetically modified soybean diet and the non-genetically modified one did not present statistical differences (P>0.05).

The biological value (BV) was higher (97.13%) for rats on the control diet and statistically different (P<0.05) to the value obtained for rats PGM_{RR} (81.42%), NGMS (82.7%) and GMS_{RR} (81.57%). According to Whitney and Rolfes (1996), a protein with a BV of 70% or more can support human growth and tissue maintenance as long as energy intake is adequate. The proteins of the PGM_{RR} diet, NGMS diet and GMS_{RR} diet could support growth and tissue maintenance. The protein nutritive value of a food reflects its ability to meet nitrogen and amino acid requirements ensuring proper animal growth and maintenance. This ability is a function of several factors, including protein content, digestibility, and amino acid composition (Cheftel et al, 1985).

The net protein utilization (NPU) value obtained for rats on diets of PGM_{RR}, NGMS and GMS_{RR} were similar (P>0.05 - 81.42%, 82.7% and 81.57% respectively),

but lower (P<0.05) than the standard protein diet (93.92%). The NPU has been suggested to be more of a practical magnitude than BV in protein quality evolution. This is due to an important and integrated part of the nutritive value of a dietary protein source. NPU is a measure of both digestibility and BV of the amino acid mixture absorbed from food (Whitney and Rolfes, 1996).

The GM_{RR} soybean and the non- GM_{RR} one present a similar protein quality according to the biological indexes NPR (1.95 to 2.19) and PRE (31.20 to 36.94) (table 5). Sikka et al (1978) found values from 2.1 to 3.5 concerning the protein in different varieties of soybean. Sarwar's studies (1997) mention the 3.7 value for protein isolated from soybean.

The protein efficiency (PRE) indicates the superiority of the casein diet over other diets, since this value to casein diet was the highest 49.36, and for test diets this ranged from 31.20 to 36.94. This was expected since casein is a pure protein source with well balanced amino acid profile and hence the choice as a standard protein diet (control diet) which other diets can be compared to.

The significantly different relations (P<0.05) between the control diet (casein) and the test diets based on tables 4 and 5 can be justified by the vegetal tissue characteristics and the presence of anti nutritional substances that limit the biological use of this feed, concerning the animal protein described by Sawar (1997). These substances or anti nutritional factors, such as the protease inhibitors (Kunitz inhibitor), can inhibit the proteolitic enzymes action when they are not correctly inactivated by the sun, leading to limitation of the protein digestion and consequently to weight gain reduction as well as animals growth (Csaky and Feneke, 2004; Liener, 1994).

Studies show that the protein value found in the soybean by the *in vitro* method present essential amino acid profiles superior to the reference standard according to FAO (1989), thus, indicating the soybean potential as source of good quality protein (Silva and Fahey Jr., 2006) and superior to the bean protein (Wu et al. 1995). Nevertheless the *in vivo* method used in literature, it can be seen differences in the values from the same protein source due to the methodological differences used as reference (Grieshop and Fahey Jr., 2006), and besides that, these methods can overestimate the protein quality from feed that have anti-nutritional factors (Sarwar, 1997). The results presented in this study can be also justified because the *in vivo* method tends to underestimate the protein quality of the leguminosas, since

the experimental models commonly used (rats in growth phase) require relatively higheramounts of sulphurated amino acids compared to human (Friedman, 1996).

It is important to emphasize that the conventional feed and the genetically modified feed must be studied case-by-case, because the molecular intervention can modify the amino acid composition, as well as the different soils can influenciate the bean nutritional composition as reported by the Grieshop and Fahey Jr. Study (2001) comparing conventional soybean cultivated in Brazil, China and The United States. The data presented here are similar to the ones reported by EFSA GMO (2008) that review the studies with conventional and genetically modified feed, showing similarities in the nutritional properties.

4. Conclusions

The biological evaluation indicates that the different diets that contain no isogenic genetically modified soybean from the producer, genetically modified soybean and the non-genetically modified isogenic soybean, also from EMBRAPA®, did not present any difference in the parameters evaluated for 28 days in Wistar rats. Protein quality of the genetically modified soybean is compared to the conventional soybean.

The research group gives attention to the publishing of these results does not mean that this study gives support to the use of genetically modified soybean.

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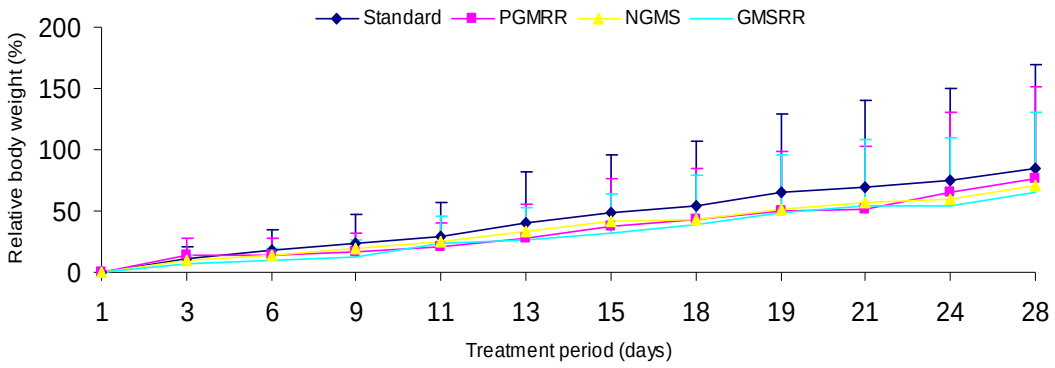
Figure 1



No significant difference (P>0.05, repeated measure ANOVA).

Figure 1 Relative weight gain of the males (with respect to day one which represented 100%) treated with standard diet, PGM_{RR} (no isogenic genetically modified soybean from productive); NGMS (non- genetically modified soybean); GMS_{RR} (isogenic genetically modified soybean).

Table 1 Composition of the experimental diets

Ingredients (%)	Casein diet	Basal diet	PGM_{RR} diet	NGMS diet	GMS_{RR} diet
Casein	12.5	-	-	-	-
PGM_{RR} flour	-	-	29.8	-	-
SE flour	-	-	-	26.5	-
GMS_{RR} flour	-	-	-	-	27.8
Sucrose	10	10	10	10	10
Soybean oil	7	7	1.3	2.3	3.9
Cellulose	5	5	5	5	5
Mineral mixture*	3.5	3.5	3.5	3.5	3.5
Vitamin mixture*	1	1	1	1	1
L - cystine	0.3	-	-	-	-
Choline bitartrate	0.25	-	-	-	-
T-butil hidroquinona	0,0014	0,0014	0,0014	0,0014	0,0014
Corn starch	60.3	73.5	49.3	51.5	48.8

* According to Reeves et al. (1993)
Casein diet, control diet = standart protein diet;
Basal diet, non-protein diet;
PGM_{RR}, genetically modified no isogenic soybean from producer;
NGMS, non-genetically modified isogenic soybean protein;
GMS_{RR} genetically modified isogenic soybean protein.

Table 2 Chemical composition (%) of diets containing genetically modified soybean and non- genetically modified*

Proximate Chemical Composition (%)	Casein diet	PGM_{RR} diet	NGMS diet	GMS_{RR} diet
Moisture	9.04 ± 0.04	8.71 ± 0.02	8.96 ± 0.01	8.57 ± 0.03
Calory	413.09 ± 0.12	336.01 ± 0.14	367.32 ± 0.11	371.97 ± 0.12
Carbohydrate**	61.38 ± 0.22	65.35 ± 0.21	64.18 ± 0.18	63.2 ± 0.18
Protein	11.18 ± 0.11	10.47 ± 0.09	11.63 ± 0.12	10.78 ± 0.07
Lipids	13.65 ± 0.06	6.97 ± 0.05	4.12 ± 0.09	8.45 ± 0.03
Fibre	2.72 ± 0.05	5.2 ± 0.02	4.63 ± 0.04	5.64 ± 0.04
Ash	2.03 ± 0.03	3.3 ± 0.02	3.48 ± 0.02	3.36 ± 0.01

Casein diet (control diet) = standard protein diet
PGM_{RR}, genetically modified no isogenic soybean from producer;
NGMS, non-genetically modified isogenic soybean protein;
GMS_{RR} genetically modified isogenic soybean protein.
*Values are means ± S.D. (standart desviation) of triplicate analysis.
**Carbohydrate = 100 – (sum of percentages of moisture, protein, lipids, fibre and ash)

Table 3 Proximate chemical composition (%) and energetic value of the ingredients used in the biological experiment*

Sample	Moisture	Energy	Protein	Lipids	Ash	Carbohydrate**
Casein	7.95	350.82	80.32	0.98	5.18	5.69
PGM_{RR}	7.21	446.09	33.54	19.05	5.08	35.12
NGMS	5.97	443.23	37.53	17.63	5.23	33.64
GMS_{RR}	6.71	408.05	35.94	11.13	5.19	41.03

* Values are means ± S.D. (standard desviation) of triplicate analysis.
PGM_{RR}, genetically modified no isogenic soybean from producer;
NGMS, non-genetically modified isogenic soybean protein;
GMS_{RR} genetically modified isogenic soybean protein.
** Total carbohydrate = 100 – (sum of percentages of moisture, protein, lipids and ash)

Table 4 Rat growth essay values* of genetically modified soybean and non-genetically modified soybean.

Parameters	Casein diet	PGM_{RR} diet	NGMS diet	GMS_{RR} diet
Body Weight gain (g)	94.46 ± 13.73[a]	72.49 ± 17.79[a]	85.85 ± 11.23[a]	78.42 ± 12.27[a]
Food intake (g)	320.33 ± 55.02[a]	349.46 ± 59.66[a]	374.98 ± 41.43[a]	386,79 ± 37.15[a]
PER	2.67 ± 0.34[a]	1.91 ± 0.22[b]	2.19 ± 0.18[b]	1.75 ± 0.27[b]
C-PER**	2.50 ± 0.32[a]	1.79 ± 0.41[b]	2.05 ± 0.16[b]	1.64 ± 0.25[b]
FER	0.30 ± 0.04[a]	0.21 ± 0.02[b]	0.23 ± 0.02[b]	0.20 ± 0.03[b]
FTI	3.40 ± 0.39[a]	4.90 ± 0.51[b]	4.39 ± 0.33[b]	5.01 ± 0.81[b]

Casein diet (control diet) = standard protein diet
PGM_{RR}, genetically modified no isogenic soybean from producer;
NGMS, non-genetically modified isogenic soybean protein;
GMS_{RR} genetically modified isogenic soybean protein.
PER (protein efficiency ratio);
C-PER (the corrected protein efficiency ratio);
FER (food efficiency ratio);
FTI (food transformation index).
*Values are means ± S.D. (standard deviations) of ten rats in each group throughout 28 days of experimental period.
**Based on value of 2.5 as standard for casein.
Means with different superscript in the same horizontal row are significantly different ($P < 0,05$).

Table 5 Nitrogen balance evalution values* of genetically modified soybean and non-genetically modified soybean.

Parameters	Casein diet	PGM_{RR} diet	NGMS diet	GMS_{RR} diet
Nitrogen consumed (g)	5.73 ± 0.98^{a}	6.28 ± 0.69^{a}	$7.2 \pm 0{,}69^{a}$	6.03 ± 1.03^{a}
Nitrogen absorbed (g)	5.49 ± 0.96^{a}	5.75 ± 0.79^{a}	6.67 ± 0.76^{a}	5.53 ± 1.02^{a}
Nitrogen retained (g)	5.33 ± 0.96^{a}	5.49 ± 0.80^{a}	6.57 ± 0.76^{a}	5.42 ± 1.02^{a}
Nitrogen faecal (g)	$0.24 \pm 0{,}04^{a}$	0.53 ± 0.18^{b}	$0.52 \pm 0{,}15^{b}$	0.49 ± 0.04^{b}
Nitrogen urinary (g)	0.16 ± 0.03^{a}	0.11 ± 0.01^{b}	0.11 ± 0.01^{b}	0.11 ± 0.01^{b}
AND (%)	95.76 ± 0.78^{a}	91.39 ± 3.45^{b}	92.65 ± 2.66^{b}	91.59 ± 1.70^{b}
TD (%)	96.69 ± 0.69^{a}	92.23 ± 3.39^{bc}	93.38 ± 2.6^{ac}	92.47 ± 1.55^{ac}
BV (%)	97.13 ± 0.66^{a}	88.28 ± 3.52^{b}	88.56 ± 0.18^{b}	88.21 ± 0.38^{b}
NPU (%)	$93.92 \pm 1{,}05^{a}$	81.42 ± 3.52^{b}	82.7 ± 2.69^{b}	81.57 ± 1.84^{b}
NPR	3.08 ± 0.31^{a}	2.1 ± 0.47^{ab}	1.95 ± 0.45^{b}	2.19 ± 0.42^{b}
PRE	49.36 ± 4.96^{a}	36.94 ± 7.0^{ab}	31.20 ± 7.21^{b}	35.03 ± 5.97^{b}

Casein diet (control diet) = Standard protein diet;
PGM_{RR}, genetically modified no isogenic soybean from producer;
NGMS, non-genetically modified isogenic soybean protein;
GMS_{RR} genetically modified isogenic soybean protein.
AND (apparent nitrogen);
TD (true digestibility);
BV (biological value);
NPR (net protein retention);
NPU (net protein utilization);
PRE (protein retention efficiency).
*Values are means ± S.D. of ten rats in each group throughout 14 days of experimental period.
Means with different superscripts in the same horizontal row are significantly different
($P < 0.05$)

ARTIGO 2

Artigo sobre a avaliação sistêmica e reprodutiva de ratos alimentados com soja geneticamente modificada

Systemic and reproductive toxicity evaluation (90 days) of genetically modified soybean treated with glyphosate in Wistar rats

Artigo formatado segundo as normas da revista Archives Toxicology.

Systemic and reproductive toxicity evaluation (90 days) of genetically modified soybean in Wistar rats

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Running title: Toxicity of genetically modified soybean in rats

Abstract

The purpose of the present study was to evaluate the possible effects of genetically modified soybean subchronical exposure in Wistar rats, both male and female, exposed during the growth period (post weaning) until the adult phase, pre and post mating, pregnancy, post pregnancy and weaning. The results point out reproductive and systemic toxicity signs in male rats exposed to any level of detectable glyphosate in the soybean diet. It is demonstrated in the weaning index that the pre and postnatal exposure to genetically modified soybean show clearly the adverse effects on the female reproductive system and the systemic effects on pups that had just been weaned. These results show that there is an association of factors that generates a probable endocrine disruption. However, other studies are necessary to elucidate the interaction mechanism that cause toxicity effects on male and female rats fed with both conventional soybean and genetically modified soybean with glyphosate residues.

Keywords: subchronic toxicity, Wistar rats, reproductive toxicity, genetically modified soybean

Introduction

The most cultivated genetically modified plant in Brazil is the soybean [*Glycine max* (L.) *Merr.*] resistant to glyphosate herbicide. There are 12.3 millions of cultivated hectares (James 2007). The genetically modified soybean resistant to glyphosate was obtained through the introduction of the gene that corresponds to the 5-enolpyruvylshikimic acid-3-phosphate synthase enzyme (EPSPS, E.C. 2.5.1.19, CP4), enzyme of the shikimic via, resistant to glyphosate, that keeps active the aromatic amino acids biosynthetic via (Busse *et al* 2001) along with the t-DNA region and the selection gene market.

The glyphosate [N-9 phosphonomethyl) glycine] acts on the EPSPS enzyme inhibiting the essential aromatic amino acid synthesis pathway, phenylalanine, tryptophan and tyrosine, which are forerunners of other products such as: lygnine, alkaloyds, flavonoids and benzoic acids (Amarante junior *et al* 2002). In principle, the genetically modified soybean resistant is tolerant to glyphosate because it has an EPSPS isoform resistant to the glyphosate molecule (Bohm *et al* 2008). The glyphosate will be absorbed and metabolized by the plant, and the plant may modify its secondary metabolism because the endogenous EPSPS enzyme is kept unaltered (Reddy *et al* 2004).

Because of the soybean genetic modification that made the soybean resistant to glyphosate, the parameters in Brazilian legislation were reviewed. In 2003, the maximum glyphosate limit permitted in beans was 0.2 mg/kg, but this limit was extended to 10 mg/kg to genetically modified soybean (Anvisa 2008). Recently studies carried out in Southern Brazil showed that the risk of contamination of soybean grains by glyphosate is possible (Bohn *et al* 2008). Those authors detected glyphosate residue and its metabolite aminometilfosfonic acid (AMPA) in high concentration in genetically modified soybean (GM_{RR}) and in the soil, interfering in nitrogen fixation (Bohn *et al* 2008).

No direct evidence that genetically modified food may represent a possible danger for health has been reported so far; however, the scientific literature in this field is quite poor and heterogeneous (Malatesta *et al* 2002). Some authors, such as Taylor *et al*. (1999) and McCann *et al* (2005), compared commercial varieties of genetically modified soybean and conventional soybean and verified that the genetic modification does not interfere in the protein, carbohydrates, fat, amino acids and

isoflavone levels. Hammmond *et al* (2004) and Zhu *et al* (2004) evaluated in rats the following parameters: ration consumption, weight gain, organs weight, blood, urine and histopathologic in corn and genetically modified soybean, respectively, resistant to glyphosate (CP4 EPSPS) during 90 days and they observed some modifications, but they did not conclude abnormal or clinical effects in organs or tissues of animals fed with the genetically modified soybean. Brake and Evenson (2004) evaluated litter size, body weight and testicular cell populations in mice the second generation during 87 days and in mice from the fourth generation during 63 days and conclude that the transgenic soybean diet had no negative effect on fetal, postnatal, pubertal or adult testicular development. Histocytochemistry of hepatocytes, pancreatic acinar, testicular cells, enzyme chemistry of serum, liver and pancreas were evaluated by Malatesta *et al* (2003, 2005), who found a significant lowering of nucleoplasmic an nucleolar splicing factors as well as a perichromatin granule accumulation in genetically modified -fed mice, suggestive of reduce post-transcriptional mRNA processing and /or nuclear export; and by Vecchio *et al* (2004), who considered possible effects in rat testes that could be associated to the herbicide that the soybean is resistant to. In some cases adverse effects were noted, which were difficult to interpret due to shortcomings in the studies (EFSA GMO 2008).

Chronical studies of glyphosate residues on food did not show weight loss, effects on pancreas or blood, or carcinogenicity evidences on human being. However, studies made with mice demonstrated weight loss, nasal discharge and death of pregnant matrices, as well as digestive disorders (Amarante Junior 2002). According to WHO (1994), studies made with technical glyphosate (as the active ingredient) in mice and rabbits, through oral administration (diet), indicate that this herbicide is not teratogenic. However, the administration of doses corresponding to 300, 1000 and 3500 mg/kg of body mass/day for female mice from 6^{th} to 19^{th} gestation day, produced growth slowing down, reduction in the number of uterinus implant and in the number of possible pups to the biggest dose WHO 1994).

Although Brazil is the third biggest worldwide producer, cultivates the genetically modified soybean for more than ten years, just being surpassed by The United States and Argentina, and it has been estimated that it represents 50% of the soybean overall production in Brazil (Embrapa, 2008), just a few studies have been conducted in the country to evaluate the biological assays, the impact of this technology on the food quality and safeness (Bohn *et al* 2008). The studies that

establish safeness or nutritional benefits should be conducted on case-by-case basis. Possible effects of the new feed resource on animal performance, animal health, efficacy, and acceptability of the new ingredient should be investigated, and time spans for such studies should be determined on a case-by-case basis (EFSA GMO 2008).

In this context we evaluated the possible effects of chronical exposure of the genetically modified soybean from the producer and from EMBRAPA® in Wistar rats, exposed during the growth period (post weaning) until the adult phase, through clinical, biochemical and histopatologic analysis, as well as reproductive indexes of male and female from paternal generation and from the second generation exposed pre and perinatal, and try to verify the possible reproductive and systemic toxicity of the genetically modified soybean in Wistar rats.

Materials and methods

Material

Genetically modified soybean (GMS_{RR}) and conventional soybean (NGMS) isogenics where obtained from Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA - Londrina, Paraná, Brazil) and genetically modified soybean no isogenic from Tupanciretã producers (PGM_{RR}) (Rio Grande do Sul, Brazil).

Detection and measurement of glyphosate residue and aminometilfosfonic acid (AMPA) in soybean

The glyphosate and aminometilfosfonic acid (AMPA) were quantified following the protocol of Veiga et al (2001). The sample preparation consisted of using 1 g of milled grains, submitted to extraction with 15 mL Milli-Q® water, kept on shaken for 30 min, placed in a sonicating for 20 min, and then centrifuged at 2000 g and 20 ºC for 20 min. Soon after, 4 mL of supernatant was extracted and filtrated in Millipore® membrane 0.45 µm. The tissue sample pellet was extracted a second time by adding 5 mL of water and the processes of sonicating, centrifuged and filtrated were

repeated. The procedures of detection and quantification followed the protocol described for Veiga et al. (2001). The quantification limits were 0.04 mg/kg for the glyphosate molecule and 0.60 mg/kg for the AMPA.

Diet preparation

Soybeans were submitted to heat treatment at 100 ºC / 5 minutes in for the possible adverse effects of protease inhibitors (trypsin and chymotrypsin) and lectins (Antunes & Sgarbieri 1980). After, the soybeans were milled, homogenized and sieved through 60 mesh. These flours were stored at cold temperature (±4ºC) for subsequent chemical analysis and for preparation of the rations.

Composition of diets

Three test diets Genetically modified soybean (GMS_{RR}) and conventional soybean (NGMS) and genetically modified soybean from Tupanciretã producers (PGM_{RR}) were prepared by mixing the soybean and other ingredients according to Reeves et al. (1993) for the growth, adult maintenance, pregnancy and lactational phases of rodents (Table 1). The standard diet was prepared according to Reeves et al (1993) but soybean was not added to the preparation (Table 1). The ingredients were homogenized and passed through a 60-mesh sieve to ensure uniform distribution of minerals and vitamins. After, the mixtures were hydrated and pelleted. The pellets were dried in an air circulation oven at 45 ºC / 12 h.

Chemical composition of grain and diets

Percentages of moisture, fat, protein, and ash were determined by the AOAC method (1996).

Animals testing

Young, 21-25 days old, white male and female Wistar rats, weighing 53 - 63 g, were obtained from the Central Animal House, Federal University of Pelotas, Rio Grande do Sul, Brazil. All breeding phases and all experiments were performed in

accordance with the rules of the Ethics and Animal Experimentation Committee. The rats were allocated divided into four groups, each consisting of ten male and ten female rats. The animals were housed individually in stainless steel metabolic cages designed for separate collection of urine. Cages were located in a room with a 12 h light/dark cycle, at a 21 ± 2 ºC of temperature, fitted with an appropriate ventilation system.

Treatment

The animals (males and females) were fed, *ad libitum*, for 90 days with the standard diet, genetically modified soybean diet and conventional soybean diet.

Males

Euthanasia

The animals were euthanized from 8 to 9 a.m. after being anesthetized with a combination of 5 mg/kg xylazine (2% xylazina chloride; Virbac®) and 90 mg/kg ketamine (5% ketamine chloride; Vetanarcol® injected intramuscularly (Allen et al. 1998). The abdomen was then incised, the vena cava was exposed, and blood was collected. Under anesthesia, the diaphragm was incised to kill the animal. Male reproductive toxicity was determined on the basis of relative weight of the reproductive organs, expressed as percentage of body weight, and of reproductive indices, including sperm number per epididymis tail, daily sperm production, sperm transit, sperm morphology and testis morphology (U.S. EPA 1996).

Systemic organs

Heart, lungs, liver, spleen, kidneys, adrenal glands and brain were carefully dissected and immediately weighed. The organ weight was related to body weight. This parameter was expressed as relative systemic organ weight.

Clinical observation

All animals were observed twice daily for mortality and once daily for overt signs of toxicity; physical examination was given weekly. Individual body weights and individual food consumption were obtained three times for week until the end of the study (week 13).

Hematology and Serum Chemistry

Hematology and seric biochemistry were carried out on all animals from each group using standard analytical methods at Laboratory of Veterinary Clinical Analysis (Faculdade de Veterinária, UFRGS, Porto Alegre, Brazil). Hematology parameters included red blood cell count (RBC), white blood cell count (WBC), hemoglobin concentration (Hb), hematocrit concentration (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and differential leukocyte count. Biochemistry parameters included total protein concentration, creatinine concentration (CRE), and alanine aminotransferase activity (ALT) were analysed.

Urine Chemistry

Urine was collected in stereo recipient at the end of 90 days in the studies on all animals from each group. Protein, pH, blood, nitrate, bilirubin, glucose, ketones and urobilinogen were assayed in urine sample with URITEST 10 INLAB Diagnostica® reagent strips.

Histopathology

All the animals in the test groups and control group were taken for histopathology studies. The internal organs (liver, kidneys, spleen, thymus, adrenal glands, heart, lungs, and brain) from each rat were dissected. Representative fragments of each organ were fixed in 10% formalin, embedded in paraffin, in order to obtain serial sections of approximately 4 μm, fixed on poly-lysine-coated glass slides, and de-paraffinised. The sections were stained with hematoxylin-eosin (H&E)

and inspected on an Olympus AX70 Routine microscope adapted with a Nikon E 4500 camera (Zeiss) for any morphological changes in the tissues due to the consumption of genetically modified soybean or conventional soybean. Photographs of representative organs from each group of rats were taken at Laboratory of Veterinary Pathology (Faculdade de Veterinária, UFRGS, Porto Alegre, Brazil). All stained slides and paraffin blocks were archived.

Sex organs

The testis, epididymis, seminal vesicle with coagulating glands (without fluid) and prostate were carefully dissected and immediately weighed. The organ weight was related to body weight. This parameter was expressed as relative sex organ weight.

Spermatid and sperm count

For spermatid number count, after removal of the tunica albuginea, each testis was minced and homogenized in 10 mL of 0.9% NaCl containing 0.5% Triton X-100 at medium speed in a FISATOM 720 tissuemizer® for one minute. After dilution in 0.9% NaCl, the number of homogenization-resistant spermatids of each testis was counted with a hemocytometer. The number of spermatids per animal (right plus left testis) was divided by 6.1 days to convert the value to daily sperm production (Robb *et al.* 1978). Similarly, the epididymis tail was cut into small pieces, minced and homogenized, and the spermatozoa were counted as described above. The epididymal sperm transit rate was calculated by dividing the epididymal sperm number by daily sperm production (Amann *et al*. 1976).

Sperm morphology

To assess the percentage of morphologically abnormal sperm (detected in the head or tail piece) the deferens ducts were rinsed with 1 mL 0.9% NaCl (for adult animals), and a sperm suspension was obtained. An aliquot of sperm suspension was carefully stained with 2% eosin to prepare a smear on the slide. Two-hundred

sperm per animal were analyzed microscopically at 400x magnification and morphologically normal and abnormal sperm were recorded according to the presence or absence of defects found in the head or tail of the spermatozoon (Robb *et al*. 1978).

Sex organs Histology

For the histological study, ten testes per group were fixed in Bouin's solution immediately after removal, embedded in paraffin, sectioned at 3 μm and subsequently stained with hematoxylin/eosin (Russell *et al.* 1990). Seminiferous tubules with complete spermatogenesis (100 tubules per testis with elongated spermatids) were analyzes microscopically at 400x magnification to assess the percentage. The presence of degenerating, sloughed and/or infiltrating cells and the absence of tubular lumen and of elongated spermatids were analyzed microscopically at 400x magnification.

Mating

One female rat was placed in a cage with one male during the dark period (between 8 p.m. and 8 a.m.) for mating. One the subsequent morning (8 a.m.), vaginal smears were obtained from all females and examined. Females showing sperm were in individual cages, and this was considered the zero day of pregnancy (Paumgartten et al. 1997). The other females were returned to the cage of the same male, each dark period during 15 consecutive days (mating period).

Maternal and reproductive outcome data

Body weight was recorded daily during the pregnancy period. The body weight noted at day zero (first period day) was considered as 100%, for each period. The differences observed during the experiment with regard to this parameter were expressed as relative weight gain. Litter size, number of living and dead pups, viable pups, and sex ratio (male/female pups) were recorded.

Females

Euthanasia

The animals were euthanized from 8:00 to 9:00 a.m. after being anesthetized with a combination of 5m/kg xylazine (2% xylazina chloride; Virbac®) and 90mg/kg ketamine (5% ketamine chloride; Vetanarcol®) injected intramuscularly (Allen et al., 1998). Under anesthesia, the diaphragm was incised to kill the animal.

Clinical observation

All animals were observed twice daily for mortality and once daily for overt signs of toxicity; physical examination were given weekly. Individual body weights and individual food consumption were obtained three times for week until the pregnancy (week 13).

Maternal variables

Body weight and food intake were recorded three times for week during pregnancy, with the value recorded on day 0 (sperm-positive smear) being considered as 100%. Maternal toxicity was characterized by decrease in relative body weight gain (expressed as percentage of body weight) and decreased in food intake, occurrence of death during pregnancy. Reproductive indices including embryo resorptions, implantation sites and pups, and pup viability were also assessed (U.S. EPA, 1996).

Pups variables

The pups variables recorded were number of pups, body weight (g), sex ratio (male/female) and occurrence of external malformations.

Statistical analysis

Parametric data (expressed as mean ± SEM) with normal distribution were analyzed by repeated measure ANOVA or one-way ANOVA, followed by the Bonferroni test when appropriate. Parametric data (expressed as mean ± SEM) as don't to present normal distribution were analyzed by Kruskal-Wallis. The non-parametric data (expressed as proportion or percentage) were analyzed by chi-square test. Differences were considered statistically significant when $P < 0.05$. The urine chemistry data were expressed by physiological or abnormal and analyzed by chi-square test (Table 2).

Results

Glyphosate levels and AMPA in the soybean

The analyses on the presence of glyphosate and AMPA in the soybean resulted in 9.6mg/kg glyphosate and 15 mg/kg AMPA of conventional soybean (NGMS) It was found 7.6 mg/kg glyphosate and 9 mg/kg AMPA in the genetically modified soybean from EMBRAPA® (GMS_{RR}) and in the genetically modified soybean from the producer (PGM_{RR}) there was not detection of glyphosate or AMPA in the limits of the method.

The amount of glyphosate consumed in the ration, NGMS and GMS_{RR} was calculated from these data. The calculation was corrected according to the amount of soybean offered during the animal's phases of life, such as the growth phase and the adult phase demonstrated on table 1.

The mean consumption of glyphosate during the growth phase was similar between the groups that received a diet prepared with NGMS and a diet with GMS_{RR} (0.5 mg/kg of rat). However, in the adult phase, the group that received conventional soybean ingested a higher amount of glyphosate than the group of genetically modified soybean (0.42 mg/kg of rat and 0.33 mg/kg of rat respectively).

Male outcome data

Animals observed during the 90 days of treatment did not present clinical signs of toxicity. At 36 days of the experiment, one animal from the group that was fed with genetically modified soybean (GMS_{RR}) died without signs of apparent toxicity. Figure 1 shows the relative weight development (^{1st} day body mass = 100%) during the period of 90 days. As expected, there was an expressive increase of relative body mass for all groups, what demonstrates variance homogeneity throughout the treatment. However, the relative corporal mass of the group that received standard diet was considerably different (P= 0.004 – ANOVA, repeated measures – Bonferroni) as compared to the groups that received diets containing soybean.

The relative food intake (Figure 2) during the 90 days of treatment showed an expressive difference (P<0.001 - ANOVA, repeated measures, followed by Bonferroni) between the group that received standard diet and the groups that received conventional soybean diet and genetically modified soybean diet (GMS_{RR}), as well as the groups that received genetically modified soybean from Producer and conventional soybean. In the 14 and 16 days (considering 3 days of evaluation) the group that received genetically modified soybean (GMS_{RR}) presented a bigger consumption increase than the other groups of treatment. Standard diet presented a decrease on the consumption in the period 22 and 29 (considering 3 days of evaluation) than the other groups of treatment.

According to the results presented on Table 3, it can be seen that there was not an expressive increase on the relative mass of brain, heart, thymus, lungs, spleen, liver, kidneys and adrenals (P = 0.05; 0.05; 0. 979; 0.430; 0.541; 0.138; 0.421, respectively, ANOVA) among the test groups and with the standard diet.

The relative mass of reproductive organs presented on Table 3, such as testis and epididymis had expressive difference. The testis of the treatment group conventional soybean presented statistical difference as compared do the standard group (P = 0.006 ANOVA, followed by the Bonferroni's test), but did not present statistical difference as compared to the test groups that contained genetically modified soybean. The relative mass of the epididymis presented statistical difference between the standard group and the treatment groups that contained soybean (P < 0.001, ANOVA, followed by Bonferroni). Seminal vesicle and prostrate

did not show any difference in their relative mass (*P* = 0.158; 0.952, respectively, ANOVA).

The lungs histopathologic analysis showed signs of toxicity, such as lymphoid hyperplasie in 5/10 animals of the groups treated with PGM_{RR} and conventional soybean (NGMS) and an incidence of 5/9 of the animals treated with genetically GMS_{RR}. The group that received standard diet (without soybean), presented incidence of 1/10 evaluated animals.

The histopatologic analysis of the reproductive organs (Table 3) did not show signs of reproductive toxicity. The hematologic analysis such as hematocrit, hemoglobin, white blood cells, red blood cells, mean corpuscular volume, mean corpuscular hemoglobin concentration, eosinophilis, basophilis and monocytes as demonstrated on Table 4, did not present expressive difference among the treated groups (*P* = 0.644; 0.487; 0.543; 0.744; 0.415; 0.574; 0.638; 0.421; 0.426, respectivally, ANOVA). The lymphocytes and neutrophilis on the group treated with conventional soybean were significativelly different in relation to the standard group (*P* = 0.015; 0.019, respectively, ANOVA – Bonferroni) according Table 4. The biochemical data such as alanine aminotransferase (ALT), creatinine and total protein, did not show statistical difference among the groups (*P* = 0.771; 0.376; 0.179, respectively, ANOVA). There were not found segmented neutrophilos NB on the analyzed samples and all sample presented platelets aggregation and at least 1+ of anisocytose and polychromasia.

The percentual of chemical parameters in the urine quality presented on Table 5, presence of protein, presence of blood, presence of nitrate, presence of bilirrubin, presence of glucose, presence of ketones, urobilinogen, pH and density determinated at the end of 90 days of treatment, did not present expressive difference among the groups (*P* = 0.2615; 1; 1; 0.3958; 1; 1; 0.1175, 1; 1 respectively, chi-square test).

Table 6 shows the fertility indices on the male rats treated with the standard diet, genetically modified soybean diets (PGM_{RR} and GMS_{RR}) and conventional soybean diet. There were observed expressive modifications on the sperm number on the group of genetically modified soybean (GMS_{RR}) (*P* = 0.002 – ANOVA followed by Bonferroni) in relation to the other groups. Although it is not expressive, it is possible to see a reduction on the daily sperm production (*P* = 0.213, ANOVA) and an increase on the abnormal sperm index (*P*>0.05 Kruskal-Wallis test) in the groups

treated with conventional soybean and genetically modified soybean (GMS_{RR}). There was not expressive statistical modification on the sperm transit rate (days) and on the tubules with complete spermatogenesis among the treated groups (*P* = 0.133; 0.138 – ANOVA, respectively).

Female outcome data

During the periods of treatment, mating and gestation there were not seen signs of apparent toxicity on the female rats.

Table 7 shows the weight gain, the food intake during the period pre-mating and the reproductive indices concerning the mating between the males and females pre-treated with standard diet, genetically modified diet (PGM_{RR} and GMS_{RR}), conventional diet (NGMS).

As it can be seen, the weight gain, the food intake and the mating index were not affected by the previous treatment (*P* = 0.291 – ANOVA; P = 0.701 - ANOVA; *P* = 0.86 – chi- square test, respectively), although, the gestation and fertility indices presented statistical difference (*P*=0.04; 0.04 respectively – chi- square test) for the female fed with genetically modified soybean (GMS_{RR}).

Table 8 shows the female relative weight gain and the relative food intake during the gestation, and the progenitor reproductive indices (fetus or pups/progenitor and lost post-implantation), evaluated at the end of the gestation. One of the progenitors of the standard group presented just one fetus and the delivery was distocio. There were not statistical differences among the groups (*P* = 0.983; 0.680 – ANOVA; *P* = 0.1629; 0.074 – respectively ANOVA and chi-square test), although it can be seen that the post-implantation lost indices of the group that received genetically modified soybean (GMS_{RR}) (27.6%) was higher than the ones of the groups that received standard diet, genetically modified soybean diet (PGM_{RR}), and conventional soybean diet (18.09%, 11.11%, 18.37% respectively). The weight gain during the gestation did not differ expressively either among the treated groups (*P* = 0.902).

The body mass at birth (*P* = 0.902, ANOVA), the pups indices such as: sex proportion, litter size, live birth index, viability index, malformation index, as well as relative weight gain and relative food intake during the breast-feeding, did not show expressive differences among the groups (Table 9) (*P*= 0.951, chi-square ; 0.991,

chi-square 0.4746, chi-square ; 0.6289, chi-square ; 2.895, chi-square ; 0.793, ANOVA; 0.905 ANOVA, respectively). In the same Table 9, two indices of the maternal and reproductive that showed expressed differences among the groups during the pregnancy and lactation can be observed. The weaning index and the number of litters of the group fed with genetically modified soybean (GMS_{RR}) presented expressive decrease (P = 0.045; 0.0047 - chi-square test) in relation to the other groups.

Discussion

The conventional soybean (9.6 mg/kg of glyphosate) and the genetically modified soybean (7.6 mg/kg) from EMBRAPA® presented levels of glyphosate residue lower than the parameter maxim allowed by the Brazilian legislation to genetically modified soybean and higher for conventional soybean . Until 2003, the glyphosate maximum limit permitted on grains was 0.2 mg/kg. This limit was extended 50 times to the genetically modified soy, changing to 10 mg/kg (Anvisa 2008). A study on the glyphosate use safeness on soybean carried out by Bohn *et al* (2008) showed that in one application of glyphosate, the concentration on the grain was 19 mg/kg, while on the grain exposed to two applications of glyphosate the concentration of glyphosate increased to 36 mg/kg. In addition, it was observed that the cultivation of soybean at the area that has been residual previous treatment with glyphosate can be result in high levels of this molecule in the grain. It could explain the presence of high level glyphosate in conventional soybean. These results are worrying once it overpass in one or two times the maximum limit on safeness established by ANVISA (Agrofit 2008). The genetically modified soybean from the producer did not present glyphosate detectable by the method (0.04 mg/kg) used in this work.

Based on the grain glyphosate levels and on the animal consumption on the groups that received conventional soybean and GMS_{RR}, it is observed that on the growth phase both groups were exposed to the same concentration of glyphosate through the diet. In the adult phase the mean concentration was lower for both groups in relation to the growth phase, but the conventional soybean group received a bigger concentration of glyphosate than the genetically modified group. Certain

stages of animal development, such as the growth, are more susceptible to the effects of toxic substances, because the organs are still being formed or maturated (Harkness and Wagner 1993).

The relative body mass gain and the relative food intake showed estatistical differences among the control group and those that received the soybean diets. However, there were not significant differences among the groups that received soybean diet. This difference observed between the standard diet and the soybean diets were expected and are related mainly to anti nutritional factors and phytate present on the soy that decreases the protein bio disposability (Sarwar 1997).

Systemic toxicity signs were not observed through relative mass of organs, but show clearly through the increase of neutrophilis and the reduction of lymphocytes (Harkness e Wagner 1993) followed by lymphoid hyperplasie in the animals'lungs fed with genetically modified soybean from PGM_{RR} and in the animals fed with conventional soybean and genetically modified soybean GMS_{RR}. Not just signs of systemic toxicity, but mainly, signs of reproductive toxicity were observed in the group that received GMS_{RR}, through an expressive increase of the tests relative mass of and expressive reduction of spermatic concentration. Effects on the reproductive system were also showed in the expressed increase of the epididymis relative mass in the groups that were fed with soybean. Concerning the other reproductive parameters, the daily production of sperm revealed a tendency of reduction to the groups that received conventional soybean and genetically modified soybean GMS_{RR}, and the time of sperm transit was delayed for the same groups, and due to a bigger variability among the animals these differences were not showed statistically.

The physiologic neutrophilia in rats is a result of the animal's agitation and stress (Garcia-Navarro and Pachaly 1998). The presence of toxic substances on the food can cause irritability, agitation and stress on the animal. The lymphocytes below the minimum normal of the species can be a consequence of hyperplasia (Garcia-Navarro and Pachaly 1998). This datum is justified by the presence of lymphoid hyperplasie in the animals that presented lymphocytopenia.

According to Amann (1982), the main variables of male toxicity are the relative mass of the tests, the testicular histology, the concentration and the spermatic morphology. The testicular histology was not affected in this assay. However, the

lack of effect on sexual organs should not used to neglect significant changes in other endpoints that may be more sensitive (Dalsenter *et al* 1999).

According to Orth (1982), the daily production and maturation of sperm can be critically dependent on the number of Sertoli cells present on the tests. These cells on the adult rat do not divide themselves and constitute a fix population.

The variability among the data can be due to the association with trigger factors. Although the toxicity mechanism is not highlighted in this study, the expositions to the genetically modified soybean and to the glyphosate show an expressive effect on the potential risk of disrupt the masculine endocrine system. Bigger doses of exposition to the glyphosate presented on conventional soybean and some reproductive parameters that are more significative in smaller doses, as in the case of genetically modified soybean GMS_{RR}, can be related to the negative feedback effect on the hormonal curve (Despopoulos and Silbernagl 1991).

A study conducted by Vecchio *et al* (2004) considers possible effects on rats'tests fed with genetically modified soybean resistant to glyphosate, the number of perichromatin granules is higher and the nuclear pore density lower. This study mentions a possible role played by traces of the herbicide to which the soybean is resistant. Dallegrave *et al* (2003) also demonstrate a set of abnormalities in the reproductive tract of the animals exposed to glyphosate-Roundup®, making evident an expressive increase on the potential risk of this pesticide in disrupts the masculine endocrine system. However, others works showed that the consumptions of GMS_{RR} treated with glyphosate did not affect these parameters (GMSO 2008).

The relative food intake and the relative weight gain of female rats treated during the period of pre-mating, lactation showed that there were not signs of systemic toxicity. However, the reproductive indices evaluated on female rats at the end of the gestation period, such as the fertility and pregnancy indices, showed that there was a decrease on the group fed with GMS_{RR}, explained by the increase on the post implantation lost of the same experimental group. Either the mechanism of hormonal interference or the sensitiveness of the sex face the hormonal alterations can be induced by an association of factors, such as the presence of glyphosate and the presence of genetically modified soybean.

The stages of implantation, organogenesis and fetal development were evaluated respectively through external mal formation indices, vitality, body mass and pups sex, showed that the progenitors pre pregnancy exposition, associated to

the exposition during the gestational period, and did not influenciate negatively these data. Making evident that the mechanisms of hormonal control on the respond-dose are activated to protect and decrease the possible effects of the endocrine disruptor (Despopoulos and Silbernagl 1991).

The teratogenic evaluation relevance on rats exposed not just during the organogenesis period, but from the male and female progenitors exposed during the pre mating, mating and the whole gestation is connected to the fact that the gametes can be modified before the fertilization and present posterior effects. Furthermore, changing on the hormonal concentrations may interfere on the maintenance and/or on the gestational development, affecting directly the sexual or general development and the fetuses viability (Kelly 1991 MacLusky and Naftolin 1981).

According to the results described, the weaning index presented an expressed reduction in the group fed with genetically modified soybean GMS_{RR}, and a tendency of reduction to the group of conventional soybean. This decrease can be associated to the mother's exposition to the glyphosate, a hydro-soluble substance (WHO 1994) that can pass through the maternal milk, or also through the exposition to the ration on pups that start the weaning from the seventeenth day and complement the diet with a ration offered to the mother.

There were observed systemic effects on male rats, as well as important effects on the reproductive system of male and female rats. Chemical and toxic agents can be interfering on the hormonal system in one or more of the following ways: (1) simulating the effects of natural hormones, linking to the receptor, (2) blocking the link of the endogenous hormone receptors, (3) reacting directly or non-directly with hormonal structures modifying them, (4) interfering on the hormone synthesis, changing the levels of hormonal receptors, (5) interfering on this hormones transport and removal (Baker 2001).

Conclusions

The results of this study showed signs of systemic and reproductive toxicity in male rats exposed to any level of glyphosate detectable in a diet of soybeans. These data are highlighted through neutrophilia, lymphocytopenia and the lungs lymphoid

hyperplasia, as well as in the epididymis and tests' relative weight and in the spermatic concentration.

The pre and postnatal exposure to the genetically modified soybean GMS_{RR} can show clearly the adverse effects on the reproductive system of females through the fertility and pregnancy indexes and in the weaning index.

These results suggest that there is an association of factors (genetically modified soybean and glyphosate) that trigger a probable endocrine disruption. However, other studies are necessary to elucidate the mechanism of interaction that causes the toxicity effects on male and female fed with conventional soybean and genetically modified soybean that presented detectable levels of glyphosate.

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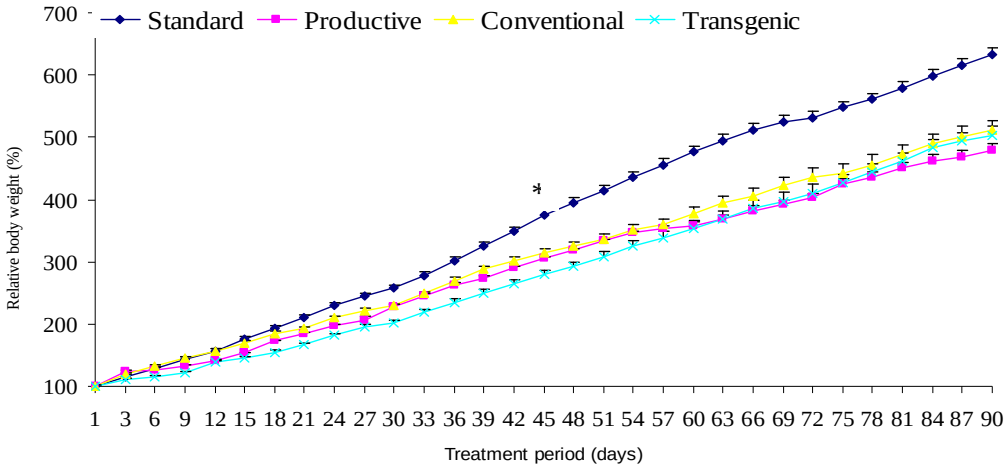
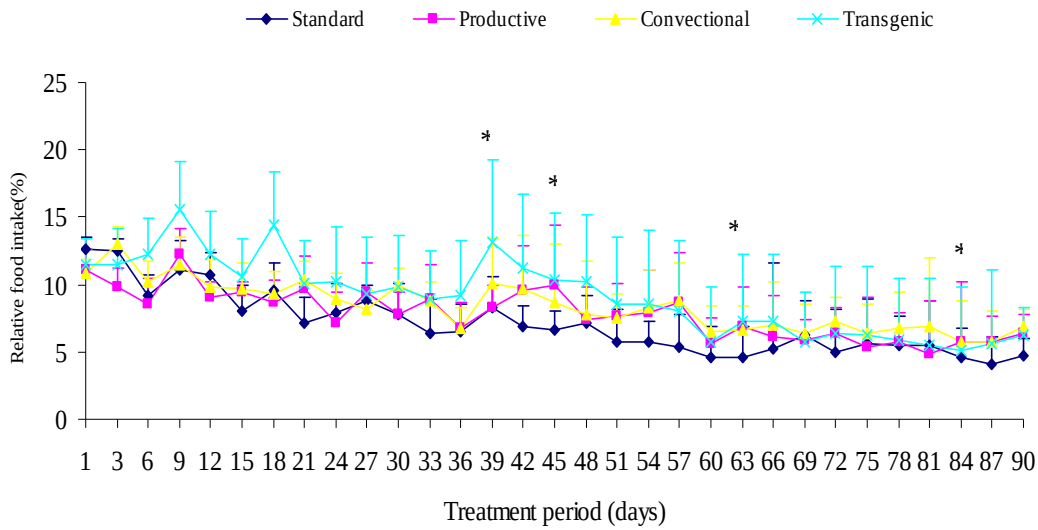


*Significant difference (P< 0.001, repeated measure ANOVA – Bonferroni test) the soybean groups to the standard since that day period.

Figure 1 Relative weight gain of the males (with respect to first day which represented 100%) treated with standard diet, PGM_{RR} diet, and conventional soybean (NGMS) diet and GMS_{RR} diet (transgenic) during 90 days of treatment. Data are reported as mean ± SEM.



*Significant difference among days 39, 45, 63, 84 (transgenic different others) (P< 0.001, repeated measure ANOVA – Bonferroni test)

Figure 2 Relative food intakes of the males treated with standard diet, PGM_{RR} diet, and conventional soybean (NGMS) diet and GMS_{RR} diet (transgenic) during 90 days of treatment. Data are reported as mean ± SEM.

Table 1 Composition of experimental diets (standard and soybean diets) for the growth, pregnancy and lactational phases of rodents (AIN-93G) and for maintenance of adults rodents (AIN-93M)

Ingredients (%)	Standard diet growth, pregnancy and lactational	Standard diet maintenance	Soybean diet growth, pregnancy and lactational	Soybean diet maintenance
Cornstarch	53.0	62.0		
Soybean flour			53.0	62.0
Casein	20.0	14.0	20.0	14.0
Sucrose	10.0	10.0	10.0	10.0
Soybean oil			7.0	4.0
Corn oil	7.0	4.0		
Cellulose	5.0	5.0	5.0	5.0
Mineral mix*	3.5	3.5	3.5	3.5
Vitamin mix*	1.0	1.0	1.0	1.0
l-Cystine	0.3	0.18	0.3	0.18
Choline bitartrate	0.25	0.25	0.25	0.25
Tert-butylhidroquinone	0.0014	0.0008	0.0014	0.0008

* According to Reeves et al. (1993).

Table2 Classification of urine qualitative variables. *

Parameters	Physiological	Abnormal
Presence of Protein (mg/dl)	30 **	31 - 500
Presence of Blood (mg/dl)	negative	positive (5 – 250)
Presence of Nitrate (mg/dl)	negative	positive
Presence of Bilirubin (mg/dl)	negative	positive (+, ++, +++)
Presence of Glucose (mg/dl)	negative or normal	positive (5 – 150)
Presence of Ketones (mg/dl)	negative	positive
Presence of Urobilinogen (mg/dl)	negative or normal	positive
pH	6 - 9	<6.0 and > 9.0
Densidade	1010 - 1045	<1010 and > 1045

* According to Garcia-Navarro (1996)

** The presence of protein in the urine is considered normal in small amounts, and it occurs mainly in the most concentrated urine.

Table 3 Relative system organs weight (%) and relative sex organs weight (%) of rats treated during 90 days* with standard diet, productive transgenic soybean diet (PGM_{RR}), conventional soybean (NGMS) diet and transgenic soybean from EMBRAPA (GMS_{RR})diet.

Parameters	**Standard** n = 10	**PGM_{RR}** n = 10	**NGMS** n = 10	**GMS_{RR}** n = 9
Brain	0.61 ± 0.08	0.74 ± 0.09	0.78 ± 0.15	0.72 ± 0.06
Thymus	0.12 ± 0.03	0.11 ± 0.04	0.12 ± 0.03	0.12 ± 0.04
Lungs	0.39 ± 0.04	0.39 ± 0.03	0.41 ± 0.03	0.39 ± 0.01
Heart	0.32 ± 0.02	0.33 ± 0.02	0.34 ± 0.02	0.33 ± 0.02
Spleen	0.22 ± 0.04	0.22 ± 0.02	0.21 ± 0.03	0.23 ± 0.03
Liver	2.83 ± 0.41	2.68 ± 0.12	2.82 ± 0.55	2.62 ± 0.19
Kidneys	0.39 ± 0.04	0.40 ± 0.47	0.40 ± 0.04	0.36 ± 0.02
Adrenals gland	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
Testis	0.57^{a} ± 0.05	0.62^{ab} ± 0.07	0.67^{b} ± 0.09	0.64^{ab} ± 0.05
Epididymis	0.18^{a} ± 0.02	0.21^{b} ± 0.02	0.22^{b} ± 0.02	0.21^{b} ± 0.01
Seminal Vesicle	0.17 ± 0.02	0.19 ± 0.03	0.20 ± 0.05	0.19 ± 0.03
Prostate	0.14 ± 0.04	0.14 ± 0.02	0.14 ± 0.03	0.13 ± 0.05

* Data are reported as mean ± SE (n = 10) based on the percentage of the organ weight in relation to total body weight.

Means with different superscript in the same horizontal row are significantly different ($P<0.05$, one way *ANOVA*- Bonferroni test).

Standard diet;

PGM_{RR} no isogenic transgenic soybean diet;

NGMS isogenic conventional soybean diet;

GMS_{RR} isogenic transgenic soybean diet.

Table 4 Hematological[*] and blood chemistry[**] evaluation of rats treated during 90 days with standard diet, productive transgenic soybean diet (PGM_{RR}), conventional soybean (NGMS) diet and transgenic soybean from EMBRAPA (GMS_{RR}) diet.[***]

Parameters	Standard n = 10	PGM_{RR} n = 10	NGMS n = 10	GMS_{RR} N = 9
Hematocrit (%)	44.6 ± 3.17	44.6 ± 3.13	46.2 ± 3.76	44.89 ± 2.67
Hemoglobin (g/dL)	14.16 ± 0.90	14.5 ± 1.17	15.16 ± 1.04	14.88 ± 0.94
WBC ($10^6/\mu L$)	3.41 ± 1.48	3.41 ± 1.38	3.45 ± 1.4	4.23 ± 1.51
RBC ($10^6/\mu L$)	8.0 ± 0.89	7.64 ± 0.64	7.85 ± 0.66	7.85 ± 0.75
MCV (fL)	56.1 ± 5.14	58.52 ± 3.47	58.93 ± 3.52	57.46 ± 3.79
MCHC (%)	32.78 ± 0.85	32.5 ± 0.90	32.86 ± 1.1	33.15 ± 1.16
Neutrophilis (%/μL)	14.7 ± 5.4[a]	20.1 ± 5.8[ab]	23.1 ± 6.62[b]	20.11 ± 4.7[ab]
Eosinophilis (%/μL)	0.5 ± 0.71	0.6 ± 0.69	0.9 ± 0.87	0.89 ± 1.05
Basophils (%/μL)	0 ± 0	0.1 ± 0.32	0 ± 0	0 ± 0
Lymphocytes (%/μL)	82.6 ± 5.76[a]	76.6 ± 5.7[ab]	73.5 ± 7.17[b]	77.3 ± 5.02[ab]
Monocytes (%/μL)	2.2 ± 1.47	2.6 ± 1.26	2.5 ± 0.97	1.67 ± 1.5
ALT (U/L)	27.38 ± 16.53	36.2 ± 29.28	32.5 ± 18.46	28.05 ± 17.12
Creatinine (mg/dL)	0.69 ± 0.11	0.80 ± 0.11	0.78 ± 0.18	0.73 ± 0.18
Total protein (mg/mL)	60.6 ± 2.32	57.2 ± 3.29	57.4 ± 5.58	59.11 ± 3.33

[*] RBC, red blood cells; WBC, white blood cells; MCV, mean corpuscular volume; MCHC, mean corpuscular hemoglobin concentration.

[**] ALT, Alanine aminotrasferase

[***] Data are reported as mean ± SEM (n = 10) throughout 90 days of experimental period.

Means with different superscript in the same horizontal row are significantly different ($P<0.05$, one way *ANOVA*- Bonferroni test).

Standard diet;

PGM_{RR} no isogenic transgenic soybean diet;

NGMS isogenic conventional soybean diet;

GMS_{RR} isogenic transgenic soybean diet.

Table 5 Frequency distribution* of urine biochemistry parameters standard diet, productive transgenic soybean diet (PGM_{RR}), conventional soybean (NGMS) diet and transgenic soybean from EMBRAPA (GMS_{RR}) diet.

Parameters*[c]	**Standard**		**PGM_{RR}**		**NGMS**		**GMS_{RR}**	
	P(%)	A(%)	P(%)	A(%)	P(%)	A(%)	P(%)	A(%)
Presence of Protein	80	20	90	10	90	10	90	10
Presence of Blood	90	10	90	10	90	10	90	10
Presence of Nitrate	80	20	80	20	80	20	80	20
Presence of Bilirubin	90	10	100	0	100	0	100	0
Presence of Glucose	100	0	100	0	100	0	100	0
Presence of Ketones	80	20	80	20	80	20	80	20
Urobilinogen	100	0	100	0	90	10	90	10
pH	100	0	100	0	100	0	100	0
Density	100	0	100	0	100	0	100	0

* P= Physiologic A= abnormal

P > 0.05, Chi square test.

Standard diet;

PGM_{RR} no isogenic transgenic soybean diet;

NGMS isogenic conventional soybean diet;

GMS_{RR} isogenic transgenic soybean diet.

Table 6 Effects of standard diet, productive transgenic soybean diet (PGM_{RR}), conventional soybean (NGMS) diet and transgenic soybean from EMBRAPA GMS_{RR} diet exposure on sperm number in the cauda epididymis, daily sperm production, sperm transit rate, percentage of seminiferous tubules with complete spermatogenesis, and sperm morphology.

Parameters*	**Standard** **n = 10**	**PGM_{RR}** **n = 10**	**NGMS** **n = 10**	**GMS_{RR}** **n = 9**
Daily sperm production (x 10^6)	11.8 ± 2.9	13.0 ± 2.3	7.5 ± 1.6	7.4 ± 2.0
Sperm number (x 10^6)	85.2 ± 9.5[a]	62.7 ± 4.3[a]	75.2 ± 5.3[a]	48.8 ± 4.0[b]
Sperm transit rate (days)	12.8 ± 3.2	5.3 ± 1.3	28.3 ± 12.0	22.4 ± 12.6
Abnormal sperm (%)[2]	6.1 ± 0.5	6.4 ± 0.8	8.2 ± 0.6	8.1 ± 0.3
Tubules with spermatogenesis (%)	99.6 ± 0.18	98.2 ± 0.49	97.9 ± 1.06	98.7 ± 0.2

* Mean ± SEM

Means with different superscript in the same horizontal row are significantly different ($P < 0.05$; ANOVA – Bonferroni test).

[2] Kruskal-Wallis test

Standard diet;

PGM_{RR} no isogenic transgenic soybean diet;

NGMS isogenic conventional soybean diet;

GMS_{RR} isogenic transgenic soybean diet.

Table 7 The reproductive indices of the female rats exposed to standard diet, no isogenic transgenic soybean diet (PGM_{RR}), conventional soybean (NGMS) diet and transgenic soybean isogenic (GMS_{RR}) diet throughout the pre-mating and mating periods

Parameters*	**Standard n = 10**	**PGM_{RR} n = 10**	**NGMS n = 10**	**GMS_{RR} n = 9**
Relative Weight gain (%)	166.35 ± 5.78	139.54 ± 5.96	137.70 ± 4.36	164.56 ± 4.46
Relative Food intake (%)	393.48 ± 58.36	396.97 ± 43.94	415.41 ± 39.40	391.36 ± 63.28
Mating index (%)[1]	100	100	100	90
Pregnancy index (%)[2]	90[a]	100[a]	100[a]	66.7[b]
Fertility index (%)[3]	90[a]	100[a]	100 [a]	66.7[b]

[1]Mating index = (number of female with sperm on vaginal smear/number of mated females) x 100.

[2]Pregnancy index = (number of pregnant females/number of females with sperm on vaginal smears) x 100

[3]Fertility index = (number of pregnant females/number of mated females) x 100.

*Means with different superscripts in the same horizontal row are significantly different (P< 0.05-Chi square test).

standard diet;

PGM_{RR} transgenic soybean no isogenic diet;

NGMS conventional soybean isogenic diet;

GMS_{RR} transgenic soybean isogenic diet.

Table 8 Reproductive indices of females.

Reproductive indices	**Standard n = 10**	**PGM_{RR} n = 10**	**NGMS n = 10**	**GMS_{RR} n = 9**
Relative weight gain (%)[1]	36.8 ± 2.1	38.7 ± 1.5	38.8 ± 1.9	40.9 ± 1.6
Relative food intake (%)	118.9 ± 36.4	123.34 ± 32.8	121.77 ± 29.5	119.17 ± 28.33
Number of pregnats	10	10	9	6
Number of pups	76	96	80	59
Pups / pregnants (mean ± SEM)	8.6 ± 1.11	9.6 ± 0.6	8.9 ± 0.59	9.8 ± 1.17
Post-implantation loss (%)[2]	18.09	11.11	18.37	27.16

[1] Relative weight gain = relative weight on the last day of pregnancy minus relative weight on the first day of pregnancy (weight on the first day of pregnancy = 100%)

[2]Post-implantation loss = (implantation sites – nº of pups/implantation sites) x 100

Standard diet;

PGM_{RR} no isogenic transgenic soybean diet;

NGMS isogenic conventional soybean diet;

GMS_{RR} isogenic transgenic soybean diet.

Table 9 Maternal and fetal outcome of dams exposed to standard diet, productive transgenic soybean diet (PGM_{RR}), conventional soybean (NGMS) diet and transgenic soybean from EMBRAPA (GMS_{RR}) diet to throughout pregnancy.

Parameters*	**Standard** **n = 10**	**PGM_{RR}** **n = 10**	**NGMS** **n = 10**	**GMS_{RR}** **n = 9**
Relative weight gain (%)	27.6 ± 1.8	30.2 ± 2.8	27.5 ± 1.9	28.3 ± 2.1
Relative food intake (%)	188.3 ± 22.6	196.6 ± 21.9	195.9 ± 19.5	189.8 ± 24.68
Litters (pups)	8 (76)	10 (96)	9 (80)	6 (59) *
Litter size (n) [1]	8.6 ±1.11	9.6 ± 0.6	8.89 ± 0.59	9.83 ± 1.28
Sex ratio (male/female)	1.1:1	0.88:1	1.03:1	1:1
Live birth index (%) [2]	86.84	98.96	81.25	98.31
Birth weight (g) [1]	5.32 ± 1.88	5.11 ± 1.81	4.98 ± 1.76	5.37 ± 1.90
Teratogenic index (%)[3]	10.53%	0	0	0
Viability index (%)[4]	96.97	89.47	81.54	96.55
Weaning index (%)[5]	95.24	81.05	70.7	62.05*

Significantly different from the control group ($P < 0.05$, chi-square test).

[1] Mean ± SEM.

[2] Life birth index = (nº of live offspring/nº of offspring delivered) x 100

[3] Teratogenic index = (nº of malformed fetuses/nº of fetuses) x 100

[4] Viability index = (nº of live pups on day 4/nº of live offspring born) x 100

[5] Weaning index = (nº of live pups on day 21/nº of live offspring born) x 100

Standard diet;

PGM_{RR} no isogenic transgenic soybean diet;

NGMS isogenic conventional soybean diet;

GMS_{RR} isogenic transgenic soybean diet.

CONCLUSÃO GERAL

Os resultados da avaliação biológica, sistêmica e reprodutiva de ratos Wistar alimentados com soja geneticamente modificada com e sem resíduo detectável de glifosato, e sem modificação genética, mas com resíduo detectável de glifosato, demonstraram:

- No ensaio de avaliação biológica a soja geneticamente modificada apresenta semelhança na qualidade protéica e na biodisponibilidade dos nutrientes em relação à soja convencional, quando consumida por ratos Wistar.
- No ensaio de toxicidade sistêmica em machos Wistar expostos por 90 dias à soja, evidenciou-se redução significativa de massa corporal relativa, consumo alimentar relativo, dados hematológicos (neutrofilia e linfopenia) e hiperplasia linfóide dos pulmões.
- No ensaio de toxicidade reprodutiva dos machos expostos a dieta com soja foi observado aumento da massa relativa dos testículos e epidídimos, e a redução da concentração espermática, revelando presença de alterações sobre órgãos e parâmetros reprodutivos após exposição durante as fases do desenvolvimento (crescimento, juvenil, peripubertal até adulto).
- A exposição por 90 dias à dieta contendo soja geneticamente modificada com resíduo detectável de glifosato induziu efeitos reprodutivos nas fases pré e perinatal das fêmeas, caracterizados pelos índices reduzidos de fertilidade e prenhez, bem como índice de desmame baixo no mesmo grupo, conferindo associação de fatores que indicam um potencial desregulador endócrino.

A partir dos resultados apresentados nos ensaios realizados, conclui-se que ratos Wistar alimentados com soja geneticamente modificada apresentam resposta biológica semelhante aos alimentados com soja não modificada geneticamente. No entanto, os ratos machos alimentados com soja geneticamente modificada ou convencional manifestaram sinais de toxicidade sistêmica e reprodutiva. Já nas fêmeas, os efeitos reprodutivos se manifestam apenas no grupo alimentado com soja geneticamente modificada contendo níveis detectáveis de glifosato.

Outros estudos são necessários para elucidar o mecanismo de interação que indica um potencial desregulador endócrino e que provoca efeitos de toxicidade em machos e fêmeas alimentados com soja convencional e soja geneticamente modificada.

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ANEXOS

Dados de catalogação na fonte:
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Biblioteca de Ciência & Tecnologia - UFPel

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