UNIVERSIDADE FEDERAL DE MATO GROSSO
FACULDADE DE NUTRIÇÃO
PROGRAMA DE PÓS-GRADUAÇÃO EM BIOCIÊNCIAS

Valor biológico da dieta à base de *okara* e os seus efeitos sobre o perfil hormonal e metabólico em ratos adultos submetidos à restrição protéica na vida intra-uterina e lactação e recuperados após o desmame

Simone Ferreira Lemes

Cuiabá-MT
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SUMÁRIO

LISTA DE ABREVIATURAS

ΔG	área sob a curva de glicose
ALT	alanina aminotransferase
AMPc	adenosina monofosfato cíclica
AST	aspartato aminotransferase
ATP	adenosina trifosfato
C	dieta controle
CC	grupo controle caseína
CO	grupo controle *okara*
CoA	coenzima A
d	dia
FER	razão de eficiência alimentar
GLUT 2	transportador de glicose 2
h	hora
HDL	lipoproteína de alta densidade
HPLC	cromatografia líquida de alta eficiência
IRS1	substrato do receptor de insulina 1
ITT	teste de tolerância à insulina
K_{itt}	constante de desaparecimento de glicose
LC	grupo recuperado caseína
LDL	lipoproteína de baixa densidade
LL	grupo hipoprotéico
LO	grupo recuperado *okara*
LP	dieta hipoprotéica
min	minuto
NPR	razão protéica líquida
PER	razão da eficiência protéica
PKA	proteína quinase A
PKC	proteína quinase C
PPAR α	receptor ativado por proliferador do peroxissoma alfa
PPAR γ	receptor ativado por proliferador do peroxissoma gama
SBCAL	Sociedade Brasileira de Ciência em Animais de Laboratório
T3	triiodotironina

TD	digestibilidade verdadeira
UCP-1	proteína desacopladora-1
UCP-3	proteína desacopladora-3
VLDL	lipoproteína de muito baixa densidade

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RESUMO

Avaliamos o valor biológico da dieta à base de *okara* e seus efeitos sobre o perfil hormonal e metabólico em ratos submetidos à restrição protéica durante a vida intra-uterina e a lactação e recuperados após o desmame. Ratos machos de mães alimentadas com dieta contendo 17% e 6% de proteína durante a gravidez e lactação foram desmamados e mantidos com dieta caseína 17% (CC, LC), dieta à base de *okara* 17% (CO, LO) e dieta caseína 6% (LL) por 60 dias. A qualidade da proteína do *okara* foi equivalente à proteína da caseína. Ingestão energética absoluta e relativa foi menor nos ratos LO e CO, mas o peso corporal não diferiu entre os grupos. Os pesos absoluto e relativo das gorduras epididimal e retroperitoneal foram menores nos grupos LO, LC e CC comparados com o grupo CO. A concentração do colesterol LDL foi menor nos ratos LO e CO comparados com os ratos LC e CC. No estado alimentado, as concentrações séricas de glicose e insulina, assim com a razão insulina:glicose foram menores nos ratos LO, CO e LC do que nos ratos CC. A concentração sérica de glucagon não diferiu entre os grupos, mas a razão glucagon:insulina foi maior nos ratos LO, CO e LC do que nos ratos CC. O conteúdo de glicogênio hepático e muscular foram menores nos ratos LO e CO do que nos ratos LC e CC. O conteúdo de gordura hepática foi maior nos ratos LO e CO do que nos ratos LC e CC. Contudo, o conteúdo de gordura hepática nos ratos LO foi menor do que nos ratos LL. A área sob a curva durante os testes de tolerância à glicose e ao piruvato foi menor nos ratos LO e CO do que nos ratos LC e CC, e o K_{itt} não diferiu entre os grupos. Portanto, a dieta à base de *okara* foi eficiente na recuperação de ratos em crescimento, previamente desnutridos e preveniu o desenvolvimento de obesidade, esteatose hepática e intolerância à glicose.

Palavras-chaves: *Okara*, desordens lipídicas, esteatose hepática, desnutrição, recuperação nutricional.

1. INTRODUÇÃO

O Brasil é o segundo maior produtor mundial de soja e estimam-se uma produção de 75,04 milhões de toneladas para a safra de 2010/11, sendo Mato Grosso o maior produtor nacional com a maior área plantada no país, 6,4 milhões de hectares. Mundialmente, apenas 7% da produção de soja são utilizados para alimentação humana e os subprodutos obtidos pelo processamento dos grãos, são geralmente descartados ou destinam-se à fabricação de ração animal (Pelaez, Albergoni & Guerra, 2004; Amaral, 2006; CONAB, 2011; Bellaver & Snizek, 2011).

A soja dá origem a diversos produtos e subprodutos, como o resíduo do seu extrato aquoso, denominado *okara* (Bowles, 2005), palavra japonesa que significa "casca honorável" (Van Der Riet, Wight, Cilliers & Datel, 1989). Segundo Cavalheiro et al. (2001), a farinha do resíduo de soja contém, em base seca, 30,3% de carboidratos, 38,0% de proteína, 12,2% de lipídeos, 16,1% de fibras e 3,4% de cinzas. Aproximadamente um terço do conteúdo de isoflavonas da soja é transferido ao *okara*. Por apresentar concentração protéica semelhante a dos grãos de soja, o *okara* apresenta um grande potencial para ser utilizado como fonte de nutrientes e isoflavonas (Jackson et al., 2002).

Apesar da alta qualidade nutricional, a digestibilidade dos produtos derivados da soja é comprometida pela presença de fatores antinutricionais e pelo processamento térmico ao qual são submetidos (Pinto & Castro, 2008). Os inibidores de proteases são os principais agentes antinutricionais presentes na soja. Outros agentes biologicamente ativos presentes são o ácido fítico, hemaglutininas, saponinas e constituintes fenólicos (Liener, 1994). A secagem é um dos tratamentos térmicos capazes de reduzir e/ou garantir que a ação destes fatores antinutricionais esteja em níveis aceitáveis e, simultaneamente, leva à diminuição da umidade, reduzindo a possibilidade de deterioração biológica e de outros mecanismos de deterioração (Singh & Heldman, 1998).

Durante o seu processamento, que consiste em tratamento térmico e secagem, o *okara* perde grande parte dos fatores antinutricionais, aumentando o valor nutricional de sua proteína (Bayram, Kaya & Öner, 2004; Aguirre et al., 1981; Pinto & Castro, 2008), que possui um bom perfil de aminoácidos (Katayama & Wilson, 2008).

Assim como outros subprodutos da soja, o uso do *okara* na alimentação humana poderia exercer efeitos fisiológicos e metabólicos preventivos ou terapêuticos em uma série de doenças crônicas, como obesidade, dislipidemias e diabetes mellitus tipo 2

(Santos, Bedani & Rossi, 2004; Hasler, 1998; Potter et al., 1998; Souza, Valle & Moreno, 2000; Matsumoto, Watanabe & Yokoyama, 2007). Devido ao seu baixo custo e elevado valor nutricional, o *okara* poderia também ser uma alternativa alimentar para a recuperação de indivíduos desnutridos em fases críticas do desenvolvimento, potencialmente propensos a desenvolverem a síndrome metabólica na vida adulta.

Em períodos críticos do desenvolvimento, o organismo é capaz de responder a situações ambientais que são estranhas ao seu desenvolvimento normal, através de adaptações moleculares, celulares e bioquímicas. Essas adaptações produzem modificações permanentes na fisiologia e no metabolismo. Assim, mesmo na ausência do estímulo que as iniciam, o organismo continua a expressar essas adaptações, e esse processo é denominado “programação metabólica” (Lucas, 1991).

Hales & Barker (1992) expandiram o conceito de “programação metabólica” e propuseram a hipótese do “fenótipo da economia” para explicar a associação entre má nutrição fetal e neonatal e doenças metabólicas na vida adulta. Segundo essa hipótese, organismos metabolicamente programados pela subnutrição intra-uterina maximizam a captação e a conservação de nutrientes. Essa estratégia garante a sobrevivência em períodos de escassez de nutrientes. Quando o suprimento alimentar é abundante, a prole com o “fenótipo de economia” acumula o excesso de nutrientes na forma de gordura predispondo-a a obesidade e outras doenças metabólicas.

Estudos em roedores têm mostrado que a má nutrição durante a vida intra-uterina e/ou lactação causa malformações nos núcleos hipotalâmicos envolvidos no controle do balanço energético (Bennis-Taleb, Remacle, Hoet & Reusens, 1999), produz alterações morfológicas e funcionais no pâncreas endócrino, especialmente das células beta pancreáticas (Desai, Crowther, Lucas & Hales, 1996; Latorraca, Carneiro, Boschero & Mello, 1998a), e nos sítios de ação da insulina (fígado, tecidos muscular e adiposo) (Desai et al., 1995; Desai et al., 1997; Latorraca, Carneiro, Boschero & Mello, 1998a). Essas alterações resultam em hiperfagia, aumento dos depósitos de gordura, dislipidemia e resistência à insulina (Zambrano et al., 2006). A incapacidade funcional das células beta pancreáticas (Latorraca et al., 1998b) e a demanda aumentada por insulina devido à obesidade e à resistência à insulina contribuem para alterações no metabolismo de carboidratos e na homeostasia glicêmica (Zambrano et al., 2006).

Em modelo de restrição protéica na vida intra-uterina e lactação, as alterações no metabolismo dos carboidratos no fígado têm sido atribuídas, ao menos em parte, ao aumento da atividade da fosfoenolpiruvato carboxiquinase e redução da atividade da

glicoquinase, indicando aumento da síntese em detrimento da utilização da glicose (Desai et al., 1995). A regulação hormonal da produção de glicose hepática também se encontra alterada nesse modelo animal. A insulina não consegue inibir a produção de glicose hepática estimulada por glucagon e esses animais também apresentam redução do número de receptores de glucagon e resistência ao hormônio. Paralelamente, esses animais apresentam aumento da concentração de receptores de insulina e de transportador de glicose 2 (GLUT 2) (Ozanne, Smith, Tikerpae & Hales, 1996a).

A resistência à insulina e as alterações no metabolismo da glicose observadas em modelo de restrição calórico-protéica na vida intra-uterina são acompanhadas por acúmulo de lipídios hepáticos e aumento da suscetibilidade ao desenvolvimento de fígado gorduroso na vida adulta. A propensão ao fígado gorduroso nesse modelo parece resultar do aumento da expressão de fatores de transcrição, de componentes da via de sinalização da insulina e de enzimas envolvidas na síntese de ácidos graxos (Thompson et al., 2007; Magee, et al., 2008; Yamada et al., 2011).

O tecido muscular e adiposo de animais submetidos à má nutrição em fases críticas do desenvolvimento exibe alterações em vários passos da via de sinalização de insulina (Latorraca et al., 1998b; Ozanne, Wang, Coleman & Smith, 1996b; Shepherd et al., 1997). Em adipócitos, embora essas alterações indiquem aumento da sensibilidade à insulina, os estudos sobre a lipólise têm mostrado que o tecido adiposo desses animais exibe certo grau de resistência ao hormônio, considerando a incapacidade da insulina inibir a lipólise em resposta ao isoproterenol (Ozanne et al.,1999; 2000).

A soja e seus subprodutos exercem efeitos benéficos sobre a homeostase energética, o metabolismo de carboidratos e de lipídios. Seus componentes (isoflavonas e proteína) parecem atuar juntos ou separadamente alterando diversos parâmetros hormonais, neuroendócrinos e metabólicos envolvidos na regulação do peso corporal, gasto energético e comportamento alimentar. A isoflavona tem efeito ambíguo sobre a ingestão alimentar (Lephart et al., 2004; Penza et al., 2006; Davis et al., 2007), mas o seu consumo invariavelmente está associado a redução do acúmulo de gordura corporal (Davis et al., 2007; Michael et al., 2006). Esse efeito parece resultar do aumento das concentrações de triiodotironina e da proteína desacopladora – 1 (UCP-1) no tecido adiposo marrom, que alteram o gasto energético e a termogênese (Lephart et al., 2004). Adicionalmente, estudos em adipócitos brancos isolados têm mostrado que a genisteína, uma isoflavona da soja, diminui a lipogênese, aumenta a lipólise e neutraliza a ação antilipolítica da insulina (Szkudelska, Nogowski & Szkudelski, 2000; 2008). Quando

incorporada a uma dieta hiperlipídica, a proteína da soja tem efeito redutor sobre a adiposidade por aumentar a capacidade termogênica dos adipócitos marrons, mediado por um aumento da expressão de UCP-1 (Torre-Villalvazo et al., 2008).

Estudos têm apresentado evidências convincentes de que a soja e seus subprodutos afetam positivamente a homeostase glicêmica, por modularem a secreção de insulina e a ação desse hormônio em tecidos periféricos. O padrão de aminoácidos da proteína da soja e as isoflavonas têm sido associados a mudanças da secreção de insulina, contudo a resposta secretória é bastante variável. Em modelo animal de hiperinsulinemia, o efeito combinado dos aminoácidos e de isoflavonas da soja produziu alterações na morfologia (redução da área das ilhotas e do conteúdo de insulina) e reduziu o transporte de glicose nas ilhotas pancreáticas e, portanto, a secreção de insulina (Noriega-López et al., 2007). Em animais hipoinsulinêmicos, a dieta a base de farinha de soja aumentou a secreção de insulina (Veloso et al., 2008). As altas concentrações de arginina presentes na proteína da soja (Torres, Torre-Villalvazo & Tovar, 2006) parecem contribuir para o aumento da insulinemia, uma vez que esse aminoácido é um potente estimulador da secreção de insulina (Fajans, Floyd, Knof & Conn, 1967). Os efeitos inibitório (Persaud, Harris, Burns & Jones, 1999) ou estimulatório (Sorenson, Brelje & Roth, 1994; Ohno et al., 1993; Jonas et al., 1995; Verspohl, Tollkühn & Kloss, 1995) da genisteína sobre a secreção de insulina têm sido atribuídos à inibição da atividade tirosina quinase (Persaud, Harris, Burns & Jones, 1999; Sorenson, Brelje & Roth, 1994) e/ou aumento da concentração de AMPc (Ohno et al., 1993) e da proteína quinase A (PKA) (Liu et al., 2006) e redução da atividade da proteína quinase C (PKC) (Persaud, Harris, Burns & Jones, 1999).

Considerando que, em roedores, o músculo esquelético representa mais de 40% da massa corporal total, o aumento da sensibilidade à insulina em resposta à soja, seus subprodutos ou componentes se deve, em grande parte, à ação da insulina no músculo esquelético (Cederroth et al., 2008; Lavigne, Marette & Jacques, 2000; Um et al., 2004). A proteína da soja é pobre em leucina, isoleucina e valina em relação à caseína, e esse perfil de aminoácidos tem sido associado ao aumento da captação da glicose e da sensibilidade à insulina no músculo esquelético (Lavigne, Marette & Jacques, 2000). Os aminoácidos de cadeia ramificada são predominantemente metabolizados no músculo esquelético e inibem a fosforilação do receptor de insulina e de seus substratos e, consequentemente, a capatação de glicose (Schwenk & Haymond, 1987; Patti et al., 1998). A genisteína também parece modular positivamente a via de sinalização da

insulina por impedir a fosforilação em serina do primeiro substrato do receptor de insulina 1 (IRS1) (Um et al., 2004). Por outro lado, a genisteína inibe a captação de glicose estimulada pela proteína desacopladora-3 (UCP-3) (Huppertz et al., 2001).

Pesquisas têm mostrado que a soja e seus derivados modulam o metabolismo de lipídios no fígado. Em ratos geneticamente propensos à obesidade, o consumo de proteína de soja reduziu o acúmulo de colesterol e de triacilglicerol no fígado, prevenindo o desenvolvimento de fígado gorduroso (Tovar et al., 2005). A redução do colesterol hepático foi associada à diminuída expressão do receptor X e seus genes alvos no fígado. A diminuição da gordura hepática tem sido atribuída ao aumento da oxidação de ácidos graxos e a redução de fatores de transcrição e enzimas chaves envolvidas na síntese *de novo* de ácidos graxos (Tovar et al., 2005; Ascencio et al., 2004). Esses efeitos são mediados, ao menos em parte, pelo aumento da razão glucagon:insulina resultante da reduzida razão lisina:arginina e aumentada concentração de glicina presente na proteína da soja (Torres, Torre-Villalvazo & Tovar, 2006).

A ingestão de soja também tem sido associada à melhora do perfil lipídico sérico, sendo a isoflavona considerada o principal agente hipolipidêmico devido à sua atividade estrogênica e antioxidante (Anthony, 2000; Polkowski, Skierski & Mazurek, 2000; Wilson et al., 2002). Em modelo animal de diabetes tipo 2, o tratamento com dieta à base de proteína de soja rica em isoflavona resultou em melhora do metabolismo de lipídios, similar aquela observada em humanos tratados com fibratos e glitazonas. Esses animais exibiram aumento da expressão das isoformas alfa e gama do receptor ativado por proliferadores do peroxissoma (PPARα e γ) (Mezei et al., 2003).

Especialmente durante a recuperação nutricional de animais submetidos à restrição protéica durante a vida intra-uterina e a lactação, uma dieta à base de farinha integral de soja teve baixa eficiência energética refletida em menor proporção de proteína na carcaça e menor massa gorda, devido em parte, a menor digestibilidade da proteína (Cheim et al., 2009). A concentração sérica de T3 e o conteúdo de UCP-1 no tecido adiposo marrom foram reduzidos, mas a capacidade termogênica não foi afetada pela dieta. A taxa de lipólise em resposta ao isoproterenol foi reduzida no adipócito branco (Paiva et al., 2011). Houve um aumento da secreção de insulina, atribuída à ação da genisteína sobre a via do AMPc/PKA (Veloso et al., 2008). Apesar do aumento da insulinemia, esses animais mantiveram-se euglicêmicos, possivelmente à custa do aumento da produção de glicose hepática, tendo em vista as baixas concentrações de glicogênio hepático (Arruda Oliveira et al., 2008). Esses animais exibiram aumento da

razão insulina:glucagon no soro e alterações na via de transdução do sinal de insulina no fígado, compatível com estado de resistência ao hormônio (Feres et al., 2010). Embora resistentes à ação da insulina, os animais exibiram diminuição da lipogênese e do acúmulo de gordura no fígado, devido à redução do conteúdo da enzima málica e ATP-citrato liase (Reis, 2007) e da expressão gênica e protéica da acetil-CoA carboxilase (Milanski et al., 2009).

O *okara* também tem se mostrado eficaz na prevenção da obesidade, esteatose hepática e desordens do metabolismo lipídico (Matsumoto, Watanabe & Yokoyama, 2007). Ratas Wistar mantidas com dieta à base de *okara* apresentaram maior fermentação fecal e diminuição do peso corporal (Préstamo et al., 2007). A suplementação da dieta com fibra dietética oriunda do *okara* aumentou a concentração de ácido butírico e a atividade antioxidante no ceco, resultando em redução do ganho de peso corporal e da colesterolemia (Jiménez-Escrig, Tenorio, Espinosa-Martos & Rupérez, 2008). O *okara* associado a uma dieta hiperlipídica reduziu a concentração sérica de lipídios e de colesterol. Esses efeitos benéficos têm sido atribuídos a sua proteína e seu teor de fibras (Villanueva et al., 2011).

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3. OBJETIVOS

3.1 Geral

Avaliar o valor biológico da dieta à base de *okara* e os seus efeitos sobre o perfil hormonal e metabólico em ratos adultos submetidos à restrição protéica na vida intra-uterina e a lactação e recuperados após o desmame.

3.2 Específicos

a. Determinar no *okara* a composição centesimal, o conteúdo de isoflavonas e o conteúdo de fibras.

b. Analisar a qualidade da proteína das dietas, através da composição aminoacídica, da razão de eficiência protéica, da razão protéica líquida, da razão de eficiência alimentar e da digestibilidade verdadeira.

c. Determinar as concentrações séricas das transaminases hepáticas, de uréia e de creatinina.

d. Estabelecer o perfil nutricional de ratos recuperados com dieta à base de *okara*, tendo como parâmetros o consumo alimentar, o peso corporal, o peso de órgãos (fígado, músculo gastrocnêmio, gordura retroperitoneal e epididimal) e as concentrações séricas de proteínas viscerais (proteínas totais, albumina e globulina).

e. Examinar o perfil hormonal de ratos recuperados com dieta à base de *okara*, através da concentração sérica de insulina e de glucagon.

f. Analisar o perfil metabólico de ratos recuperados com dieta à base de *okara*, verificando a glicemia de jejum e pós-prandial, a tolerância à glicose durante o teste intraperitoneal de tolerância à glicose, a produção de glicose durante o teste intraperitoneal de sensibilidade ao glucagon nos estados de jejum e pós-absortivo e a produção de glicose durante o teste intraperitoneal de tolerância ao piruvato nos estados de jejum e pós-absortivo.

g. Determinar o perfil metabólico, analisando as reservas de glicogênio hepático e muscular nos estados de jejum e pós-absortivo e o conteúdo de gordura hepática.

4. ARTIGO

Nutritional recovery with *okara* diet prevented obesity, hepatic steatosis and glucose intolerance

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ABSTRACT

We assessed the biological value of an *okara* diet and its effects on the hormonal and metabolic profile of rats submitted to protein restriction during intra-uterine life and lactation and recovered after weaning. Male rats from mothers fed either 17% or 6% protein during pregnancy and lactation were maintained on 17% casein (CC, LC), 17% *okara* (CO, LO) or 6% casein (LL) diets over 60 days. The nutritional quality of the *okara* protein was similar to that of casein. Total and relative energy intake was lower in LO and CO rats, but body weight did not differ among the groups. The absolute and relative weights of epididymal and retroperitoneal fat deposits were lower in LO, LC and CC groups than in the CO group. In the fed state, serum glucose and insulin concentrations and the insulin:glucose ratio were lower in LO, CO and LC rats than in CC rats. Serum glucagon concentrations did not differ among groups, but the glucagon:insulin ratio was higher in LO, CO and LC rats than in CC rats. Liver and muscle glycogen contents were lower in LO and CO rats than in LC and CC rats. Liver fat content was higher in the LO and CO rats than in LC and CC rats. However, liver fat content in LO rats was lower than in LL rats. The glucose area under the curve during glucose and pyruvate tolerance tests was lower in LO and CO rats than in LC and CC rats. Thus, *okara* diets were effective in the nutritional recovery of early malnourished rats and also prevented the development of obesity, hepatic steatosis and glucose intolerance.

Keywords: Okara, lipid disorders, steatosis, early malnutrition, nutritional recovery

INTRODUCTION

Okara is a byproduct of soy milk and is rich in protein, lipids and dietary fiber (Jiménez-Escrig, Tenorio, Espinosa-Martos & Rupérez, 2008). *Okara* protein has a good essential amino acid profile and *in vitro* digestibility (Ma, Liu & Kwok, 1997; Chan & Ma, 1999), and its lipids are predominantly polyunsaturated. Small amounts of starch, sugars, potassium, and significant levels of B vitamins are also present (Van der Riet, Wight, Cilliers & Datel, 1989). In addition, approximately 1/3 of the isoflavones present in the soybean remains in *okara* (Bowles & Demiate, 2006; Jackson et al., 2002), suggesting that it is a good, low-cost source of nutrients for human and animal nutrition.

Okara has been shown to be effective in preventing obesity, hepatic steatosis and lipid metabolism disorders (Matsumoto, Watanabe & Yokoyama, 2007). When used as a supplement to enrich dietary fiber in rats, *okara* had no influence on food intake, reduced growth rate and feeding efficiency, and increased the total short chain fatty acid production (Préstamo et al., 2007). O*kara*, when combined with a high-fat diet, reduced total lipids and cholesterol concentrations in serum from rodents (Villanueva et al., 2011). Suppression of the increase in plasma cholesterol levels in a hypercholesterolemic rat model was observed after a four-week treatment with *okara* (Fukuda et al., 2006). The beneficial effects of *okara* have been attributed to its protein and fiber content (Villanueva et al., 2011).

Due to its reduced cost, elevated nutritional value and beneficial effects on obesity, diabetes and/or lipid disorders, *okara* could be used as an alternative feed for recovering from early-life malnutrition, which is a risk factor for metabolic syndrome.

The aim of this study was evaluate the biological value of an *okara* diet and its effects on the hormonal and metabolic profile of a rat model submitted to protein restriction during intra-uterine life and lactation and recovery after weaning.

MATERIALS AND METHODS

Animals and diets

The experimental procedures involving rats were performed in accordance with the guidelines of the Brazilian Society of Science in Laboratory Animals (SBCAL) and were approved by the ethics committee at the Federal University of Mato Grosso (process Nº 23108.019180/10-3). Male and virgin female Wistar rats (85-90 days old) were obtained from the university's breeding colony. Mating was performed by housing

male rats with female rats overnight (one male and five female rats), and pregnancy was confirmed by the examination of vaginal smears for the presence of sperm. Pregnant females were separated at random and maintained from the first day of pregnancy until the end of lactation on isocaloric diets containing 6% (low protein [LP] diet) or 17% protein (control [C] diet). Spontaneous delivery took place at day 22 of pregnancy after which, at 3 days of age, large litters were reduced to eight pups to ensure a standard litter size per mother. After weaning (twenty-five days), the males were divided into five groups: CC, consisting of offspring born to and suckled by mothers fed a C diet and subsequently fed the same diet after weaning; CO, consisting of offspring born to and suckled by mothers fed a C diet and subsequently fed an *okara* diet with 17% protein after weaning; LL, consisting of offspring of mothers fed an LP diet and subsequently fed the same diet after weaning; LC, consisting of offspring of mothers fed an LP diet and a C diet after weaning; and LO, consisting of offspring of mothers fed an LP diet and fed an *okara* diet containing 17% protein after weaning.

The *okara* was produced in the laboratory from soybeans (*Glicine max*) obtained from a local market in Cuiabá, Brazil. Preparation of *okara* was performed according to the Lescano et al. method (2005), with minor modifications. The soybeans were cleaned, washed with tap water, cooked for 5 minutes (thermal process), drained and washed again with tap water. Water was added to the beans (ratio of 1 kg of soybean/5 L of water), which were then cooked for an additional 5 minutes, after which they were crushed. The next stage was to cook the soybeans at 100 °C for 10 minutes to elaborate the soymilk, followed by a sifting process to extract the *okara*. The *okara* extract was then dried in an oven at a temperature of 55 °C and ground to a powder. The composition of the o*kara* was analyzed using established methods. The moisture content of the *okara* was determined by weight loss after oven-drying at 105ºC to a constant weight. Ashes were determined by incineration (at 550ºC for 4 hours) in a muffle. Nitrogen content was determined by the micro-Kjeldahl method, using a nitrogen conversion factor of 6.25 (AOAC, 1995). The fat content was determined by extraction with petroleum ether using the soxhlet method (James, 1995). Fiber content was determined by an enzyme-gravimetric method according to Proscky et al. (1984). The carbohydrate content was calculated by subtracting protein, fat and fiber content from *okara* weight.The composition of the *okara* and other diets are presented in Table 1. In the *okara* diet, adjustments were made to equalize protein and lipid content to the casein diet, suppressing soybean oil and fiber and reducing carbohydrates. The control

and *okara* diets were isonitrogenous, with 17% protein composition, prepared in accordance with the recommendations of the American Institute of Nutrition AIN-93G (Reeves, Nielsen & Fahey, 1993). The diets' amino acid composition was determined by high-performance liquid chromatography (HPLC) (Paucar-Menacho et al., 2010; White, Hart & Kry, 1986). The isoflavone content of dry *okara* was also determined by HPLC (Berhow, 2002; Murphy, Barua & Hauck, 2002).

Biological methods for evaluation of diets

Biological protein quality was determined by the protein efficiency ratio (PER) and the net protein ratio (NPR). The PER consisted of the ratio between the animals' weight gain and protein consumption during twenty-eight days. The NPR adds to body weight gain in the protein diet group, whereas it adds to weight loss in the group fed the protein-free diet. Additionally, the true digestibility (TD) and food efficiency ratio (FER) were determined. The TD was determined using the following formula: TD= [nitrogen intake – (fecal nitrogen – metabolic fecal nitrogen)]/nitrogen intake x 100 (Gilani & Sepehr, 2003). Fecal nitrogen was measured by the micro-Kjeldahl method (AOAC, 1995). The FER was considered to be the animals' weight gain and the cumulative consumption of food for twenty-eight days.

At 23 days of age, eighteen male rats newly weaned male rats were divided into three groups: 6 animals fed a casein diet, six animals fed an *okara* diet (both diets were isonitrogenous with 10% protein for twenty-eight days), and six animals fed a protein-free diet for 12 days. These diets were balanced and prepared in accordance with the recommendations of the American Institute of Nutrition-AIN-93G (Reeves, Nielsen & Fahey, 1993). The rats were kept in individual cages maintained at constant temperature (24°C) and controlled lighting (12/12-h light-dark cycle). Water and food was offered *ad libitum*.

Determination of the metabolic, hormonal and biochemical profiles

To evaluate metabolic, hormonal and biochemical profiles, offspring were weaned at the 4th week after birth. They were kept in collective cages (4 to 5 animals per cage) under standard lighting conditions (12/12-h light-dark cycle) at a temperature of 24°C. Throughout the experimental period, the rats were given free access to food and water. Food intake and body weight were recorded three times per week.

Glucose tolerance test

At 90 days and after a 12-h fast, the rats were administered glucose (200 g/L) subcutaneously at a dose of 2 g/kg of body weight. Blood samples were obtained from the cut tail tip 0, 30, 60 and 120 min later to determine serum glucose concentrations (Accu-Chek® portable glucose meter, Roche Diagnostics, Germany). The glucose response during the glucose tolerance test was calculated by calculating the total area under the glucose (ΔG) curve using the trapezoidal method (Matthews, Altman, Campbell & Royston, 1990).

Insulin tolerance test

At 90 days and after a 12-h fast, the rats were administered insulin (regular) intraperitoneally at a dose of 1.5 U/kg of body weight. Blood samples were obtained from the cut tail tip 0, 5, 10 and 15 min later to determine serum glucose concentrations (Accu-Chek® portable glucose meter, Roche Diagnostics, Germany). The glucose response during the insulin tolerance test was evaluated by the rate constant of plasma glucose disappearance (K_{itt}), which was calculated from the slope of the fall in log-transformed plasma glucose between 0 and 15 minutes (Lundbaek, 1962) after insulin administration when the glucose concentration declined linearly.

Pyruvate tolerance test

For the pyruvate tolerance test, rats were fed or fasted for 12 h with free access to water before intraperitoneal injection of 2 g/kg sodium pyruvate (Sigma). Blood samples were obtained from the cut tail tip before and at 15, 30, 60, 90, 120 and 150 minutes after sodium pyruvate injection. Blood glucose was determined using the Accu-Chek® Active glucometer (Roche). Glucose response during the pyruvate tolerance test was calculated by estimating the total area under the glucose curve (ΔG), using the trapezoidal method (Matthews, Altman, Campbell & Royston, 1990).

Glucagon sensitivity test

For the glucagon sensitivity test, the rats were fed or fasted for 6 h and 12 h with free access to water before intraperitoneal injection of 0.2 mg/kg human glucagon (Sigma). Blood samples were obtained from the cut tail tip before and 5, 10, 20 and 40 minutes after glucagon injection. Blood glucose was determined using the Accu-Chek® Active glucometer (Roche). Glucose response during the glucagon sensitivity test was

calculated by estimating the total area under the glucose curve (ΔG), using the trapezoidal method (Matthews, Altman, Campbell & Royston, 1990).

Biochemical and hormonal profile

The rats were decapitated, blood samples were collected, serum was obtained by centrifugation, and aliquots were used to measure serum urea, serum creatinine, total serum protein, serum albumin, serum globulin, serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST) levels and lipid profile (BT-3000 Plus, Wiener Lab, Rosario, Argentina). Serum insulin and glucagon concentrations were determined using the Milliplex Map Rat Endocrine Panel (Millipore) according to the manufacturer's instructions.

Organ weights

After medial laparotomy, *gastrocnemius* muscle, retroperitoneal and epididymal white adipose tissue were quickly removed and their fresh weights determined. Livers were removed and weighted, and aliquots were frozen immediately in liquid nitrogen and stored at -80ºC to determine hepatic fat (Folch, Lees & Sloane Stanley, 1957) and glycogen (Carroll, Longley & Roe, 1956) content. The hind limb (*gastrocnemius*) was excised to determine its glycogen content (Carroll, Longley & Roe, 1956).

Statistical analyses

The results were expressed as the mean ± standard deviation for the number of rats indicated. The casein and *okara* diets were compared with the unpaired Student's-*t* test. Two-way analysis of variance (effects of nutritional status and diet) was used to compare data from the CC, CO, LC and LO groups. The same data were analyzed by one-way analysis of variance when assessing whether diet was effective in improving the nutritional status in the LC, LO and LL groups. When necessary, these analyses were complemented by the least significant difference test to determine the significance of individual differences. Levene's test for homogeneity of variances was initially used to determine whether the data complied with the assumptions for parametric analysis of variance. $P<0.05$ indicated statistical significance. All statistical comparisons were performed using the STATISTICA software package (StatSoft Inc., Tulsa, OK, USA).

RESULTS

The *okara* diet possesses a higher content of glycine, arginine, alanine, aspartic acid, phenylalanine and isoleucine and a lower content of proline, methionine, cysteine, cysteine, histidine, glutamic acid, tyrosine, valine and lysine, when compared with the control diet. Levels of all amino acids in the *okara* diet were higher than in the low-protein diet (Table 2).

The total isoflavone concentration for dry *okara* was 100.5 mg/100 g. The composition of isoflavones was predominantly biologically inactive isoflavone glucosides of genistin, daidzin and glycitin (43.3 mg/100 g). The isoflavone aglycone composition was 15.5 mg/100 g, with higher amounts of genistein (Table 3).

The FER in the *okara* diet was increased by 82% compared with the casein diet, whereas PER, NPR and TD were similar in both groups (Table 4).

Weight gain during the recovery phase was lower in LC and LO rats compared with CC and CO rats ($F_{1,84}$=51.32, $p<0.001$) but was higher in CO and LO rats than in CC and LC rats ($F_{1,84}$=7.60, $p<0.01$). In recovered rats (LO and LC groups), the weight gain was similar and both groups showed higher weight gain than LL rats ($p<0.001$). At the end of the recovery period, body weights of rats from LC and LO groups were lower than those from CC and CO groups ($F_{1,\ 84}$= 74.84, $p<0.001$). Additionally, the final body weights of rats from LO and LC groups were equal, and both exhibited final body weights higher than LL rats ($p<0.001$). The absolute energy intake was lower in the LO group than in the LC, CO and CC groups ($p<0.01$). The total energy intake was decreased in the CO group compared with the CC group ($p<0.01$). When expressed per 100 g of body weight, the energy intake was lower in recovered rats than in control rats ($F_{1,\ 76}$= 4.89, $p<0.05$), as well as in rats fed with the *okara* diet, compared with those fed the casein diet ($F_{1,76}$= 127.34, $p<0.001$). In LO rats, the absolute energy intake was higher than in LL rats and lower than in LC rats. The relative energy intake in LO rats was lower compared with LC and LL rats ($p<0.001$) (Table 5).

The absolute weight of livers and muscle were lower in recovered rats than in control rats ($F_{1,44}$=10.56, $p<0.01$ and $F_{1,71}$=14.87, $p<0.001$, respectively). When expressed per 100 g of body weight, liver weights were similar in rats fed the *okara* diet, and both groups were lower in relation to CC rats ($p<0.001$). The relative weight of muscle was similar in all groups. In recovered rats, the absolute weight of livers and muscle were similar but higher than in LL rats ($p<0.001$). When expressed per 100 g of body weight,

liver weights were similar between LO and LC , and both groups had lower liver weights than LL rats ($p<0.001$). The absolute and relative weights of epididymal and retroperitoneal fat deposits were lower in the LO, LC and CC groups compared with the CO group ($p<0.01$). In the recovered groups, the absolute weights of epididymal and retroperitoneal fat deposits were higher compared with the LL group ($p<0.001$). The relative body weight of the epididymal fat deposits was lower in LO and LC rats compared with LL rats ($p<0.01$). No difference was observed in the relative weight of retroperitoneal fat deposits in the LC, LO and LL groups (Table 6).

Serum urea concentration was higher in recovered rats than in control rats ($F_{1,18}= 11.00$, $p<0.01$) and was higher in rats fed the *okara* diet than in those maintained with the casein diet ($F_{1,18}= 16.01$, $p<0.001$). In LO rats, the serum urea level was higher than in LC and LL rats ($p<0.01$). Serum creatinine concentrations did not differ between the CC, CO, LC and LO groups. In LO and LL rats, serum creatinine concentrations were higher compared with LC rats ($p<0.01$). Serum total protein concentrations were higher in recovered rats than in control rats ($F_{1,18}=7.70$, $p<0.05$). In LO and LC rats, serum total protein concentrations were higher than in LL rats ($p<0.01$). Serum albumin concentrations were higher in LO rats than in CC and CO rats, as well as in LC and CO rats, compared with CC rats ($p<0.05$). In recovered rats, serum albumin concentrations were higher than in LL rats ($p<0.01$). No differences were observed in serum globulin concentrations between the CC, CO, LC and LO groups. In LO rats, serum globulin concentrations were similar to both LC and LL rats. Serum total cholesterol concentrations in the LO group were similar to those in the CO and CC groups and lower compared with the LC group ($p<0.01$). Serum low-density lipoprotein cholesterol (LDL-cholesterol) concentrations were lower in LO and CO rats compared with LC and CC rats ($F_{1,7}=5.83$, $p<0.05$). In LO rats, serum high-density lipoprotein cholesterol (HDL-cholesterol) concentrations were lower than in LC and CC rats but similar to CO rats ($p<0.05$). Total serum cholesterol, LDL and HDL concentrations were lower in the LO group compared with the LC and LL groups ($p<0.05$). No differences were observed among the groups in the concentrations of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT), triglycerides and very low-density lipoprotein (VLDL) (Table 7).

In the fasting state, neither previous nutritional status nor the *okara* diet, or interaction between these factors, interfered with the fasting serum glucose, insulin and glucagon

concentrations or the insulin:glucose and glucagon:insulin ratios. Serum insulin concentrations in LO rats were similar to both LC and LL rats. The insulin:glucose ratio in LO and LL rats was similar and lower compared with LC rats ($p<0.05$). In the fed state, serum glucose and insulin concentrations, as well as the insulin:glucose ratio, were lower in CO, LC and LO rats compared with CC rats ($p<0.01$). In LO and LC rats, the serum insulin and insulin:glucose ratios were higher than in LL rats ($p<0.05$). Serum glucose concentrations were similar in LO, LC and LL rats. The serum glucagon concentrations were similar among all groups. The glucagon:insulin ratio was higher in LO, CO and LC rats than in CC rats ($p<0.05$). In recovered rats, the glucagon:insulin ratio was lower than in LL rats ($p<0.05$) (Table 8).

In the fasting state, liver and muscle glycogen content did not differ among CC, CO, LC, LO and LL groups. The liver fat content was higher in rats fed the *okara* diet than in those fed the casein diet ($F_{1,17}=10.23$, $p<0.01$). In recovered rats, liver fat content did not differ but was lower than in LL rats ($p<0.05$). In the fed state, liver and muscle glycogen content was lower in rats fed *okara* than in those fed with the casein diet ($F_{1,19}=16.82$, $p<0.001$ and $F_{1,15}=7.86$, $p<0.05$). In LO rats, liver glycogen content was lower than in LC and LL rats ($p<0.05$) that exhibited similar values. In the LC and LO groups, muscle glycogen content was equal, and both groups had lower muscle glycogen content than the LL group ($p<0.05$). The liver fat content was higher in rats fed the *okara* diet than in those fed the casein diet ($F_{1,17}= 17.10$, $p<0.001$). In the LO and LC groups, liver fat content did not differ, but both groups showed values lower than in LL groups ($p<0.05$) (Table 9).

The mean total areas under the curves in response to glucose load were similar in the rats fed the *okara* diet. However, LO rats exhibited higher ΔG than LC rats, whereas CO rats had lower ΔG than CC rats. In LO rats, the ΔG was higher than in LC and LL rats ($p<0.001$) (Figure 1A). The rate constant for serum glucose disappearance (K_{itt}) during the insulin tolerance test (ITT) did not differ among the five groups (Figure 1B).

After 6 h of fasting, the mean total areas under the glucose curves during the glucagon sensitivity test were higher in recovered rats than in control rats ($F_{1,12}=8.44$, $p<0.05$). After 12 h of fasting, ΔG during the glucagon sensitivity test did not differ among groups (data not shown). In LC and LO rats, the mean total areas under the glucose curves during the glucagon sensitivity test was higher than in LL rats ($p<0.001$) (Figure 1C). In the fed state, the mean total areas under the glucose curves during the glucagon

sensitivity test was higher in rats maintained on the *okara* diet than in those fed the casein diet ($F_{1,12}$=12.27, $p<0.01$). In LC and LO rats, the ΔG during the glucagon sensitivity test was higher than in LL rats ($p<0.01$) (Figure 1D).

After 12 h of fasting, the mean total areas under the glucose curves during the pyruvate tolerance test were lower in CO and LC rats than in CC rats ($p<0.05$). In the LO group, ΔG was similar to CC, CO and LC groups (Figure 1E). In the fed state, the mean total areas under the glucose curves during the pyruvate tolerance test were lower in rats fed the *okara* diet than in rats maintained with the casein diet ($F_{1,14}$=5.28, $p<0.05$). No difference was observed among the LC, LO and LL groups (Figure 1F).

DISCUSSION

As expected, the *okara* diet provided lower nutritional availability of some essential amino acids, especially cysteine, cysteine and methionine, compared with the casein diet. Additionally, the rats maintained on the *okara* diet exhibited higher serum concentrations of urea than those maintained on the casein diet. There is a good correlation between plasma urea concentration and protein quality (Eggum, Beames, Wolstrup & Bach Knudsen, 1984), and it has been shown that soy protein is proportionally more degraded to urea than casein protein (Deutz, Bruins & Soeters, 1998). However, in this study, the quality of *okara* protein seemed to be equivalent to that of casein, as judged by the similar PER and NPR values, better weight gain and similar final body weight, despite lower absolute and relative energy intake that resulted in a higher FER. The *okara* protein quality was also confirmed by the unaltered activity of hepatic enzymes (ALT and AST) and normal serum visceral proteins levels, that is marker nutritional status. Even when consumed as the only source of protein, *okara* appeared to meet the protein requirements of growing rats that were previously malnourished. It is possible that the minimization of the anti-nutritional factors by thermal treatment contributed to efficient digestion and improved bioavailability of the *okara* protein.

Weight gain does not necessarily reflect protein accretion, and some diets can cause water retention and/or increase fat deposits. In the present study, protein accretion seemed adequate because the weight of *gastrocnemius* muscle and serum creatinine levels were similar in all groups. Creatinine is a metabolite of phosphocreatine found in muscle, which shows little variation and is related to muscle mass (Schutte et al., 1981).

A soybean diet has been associated with a reduction in fat deposits due to the action of isoflavones, which increase energy expenditure by altering the activity of brown adipose tissue and thyroid function (Lephart et al., 2004; Takahashi & Ide, 2008). Moreover, it has been shown that the soybean diet's lower methionine content and lysine/arginine ratio, when compared with casein, reduces metabolizable energy intake, total energy and fat gains, without significantly modifying the total energy intake in rats maintained on the soybean diet with an additional cornstarch diet (Hurley, Richard, Deshaires & Jacques, 1998). The *okara* diet used in the present study contained considerable amounts of isoflavones and had a lower lysine/arginine ratio but produced diverse effects on fat deposit weight. For rats subjected to protein restriction in early life, the *okara* diet did not alter the retroperitoneal and epididymal fat weight, whereas for the offspring of control rats, the same diet increased retroperitoneal and epididymal fat weight. We previously showed the unaltered thermogenic capacity of brown adipose tissue in rodents maintained on a soybean flour diet. The proportions of fat deposits are determined by the lipolysis rate, which differed depending on the previous nutritional status (Paiva et al., 2011).

There is a positive association between fat body mass and serum triglycerides concentration (Nakanishi et al., 2000). It has also been shown that soy protein consumption prevents triglyceride accumulation in the liver, decreasing the deleterious effects of lipotoxicity (Ascencio et al., 2004). Dietary fiber, soy protein and isoflavones exert beneficial effects on serum cholesterol levels (Torres, Torre-Villalvazo & Tovar, 2006; Villanueva et al., 2011). In the present study, the *okara* diet did not alter serum triglycerides concentrations but reduced serum LDL-cholesterol levels, independently of the body and fat weight or previous nutritional status. The high *okara* fiber content and low serum insulin levels may contribute to the reduction of serum LDL-cholesterol levels because insulin is directly associated with lipoprotein, and dietary fiber is inversely associated with serum cholesterol concentrations (Orchard et al., 1983; Capaldo et al., 1985; Jenkins & Jenkins, 1984).

In general, soybean protein possesses a high arginine:lysine ratio and glycine content, and this amino acid profile enhances serum glucagon and reduces the serum insulin level, thus increasing the serum glucagon:insulin ratio (Torres, Torre-Villalvazo & Tovar, 2006). In the present study, the *okara* diet increased the serum glucagon:insulin ratio, despite no modification of serum glucagon levels. Curiously, although this hormonal profile points to catabolic (lipolysis) instead of biosynthetic (lipogenesis)

processes, we observed enhanced fat liver content, especially in the offspring of control rats. In the recovered rats, the *okara* diet prevented typical fat deposition observed in malnutrition. It is possible that the increased serum glucagon:insulin ratio produced excessive mobilization of body fat that was stored in the liver. It is also conceivable that increased short chain fatty acids (especially acetate and propionate), derived from elevated fiber content in the *okara* diet, may be involved in fat accumulation in adipose tissues and livers. This assumption was based on the fact that acetate and propionate stimulate fat accumulation in liver and adipose tissue (Miyazaki et al., 2001; Hong et al., 2005).

The glucagon:insulin ratio and the availability of substrates are determinants for the metabolic directions in glycogenolysis/glycogenesis and gluconeogenesis/glycolysis and are therefore important contributors to metabolic homeostasis. Our findings are consistent with these statements. To obtain isonitrogenous diets, the carbohydrate content was reduced in the *okara* diet, which, combined with its high fiber content, may have contributed to reduced serum insulin levels and a high glucagon:insulin ratio in the fed state. In rats maintained on the *okara* diet, the low insulin:glucose ratio in the fed state, and the areas under glucose curves for the glucose tolerance test and the pyruvate tolerance test indicate higher insulin sensitivity. However, the K_{itt} value and the metabolic parameters in the fasting state indicate normal insulin sensitivity. Because pyruvate (together with citrate) is the main source of hepatic glucose production after a long fasting period, it is possible that the *okara* diet was able to block gluconeogenesis in the fasting state. It is also reasonable to hypothesize that, in the fed state, the low area under the glucose curves during the pyruvate tolerance test reflects a shift of hepatic glucose produced by glycogen synthesis favoring the reposition of low glycogen reserves. Low glycogen reserves can be partially responsible for a relatively reduced liver weight. The unaltered area under the glucose curves, during the glucagon sensitivity test at 6 h and 12 h of fasting, indicate normal glucagon sensitivity in rats fed an *okara* diet. In the fed state, the increased glucagon:insulin ratio was reinforced by the glucagon challenge and resulted in a high ΔG.

CONCLUSION

Our results indicate that the *okara* diet was effective in the nutritional recovery of growing rats who suffered early malnutrition. Moreover, the *okara* diet prevented

obesity, hepatic steatosis and glucose intolerance in an animal model prone to diabetes mellitus.

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HIGHLIGHTS

We analyzed the chemical composition of an *okara* diet.

We determined the amino acid and isoflavones profile of an *okara* diet.

We examined the biological value of an *okara* diet.

We evaluated glucose tolerance and hepatic glucose output in rats recovered with an *okara* diet.

We evaluated the insulin and glucagon sensitivity in rats recovered with an *okara* diet.

We determined the glycogen and fat in the liver of rats recovered with an *okara* diet.

ABBREVIATIONS: protein efficiency ratio (PER), net protein ratio (NPR), fed efficiency ratio (FER), true digestibility (TD), total area under the glucose curve (ΔG), rate constant for the serum glucose disappearance (K_{itt}), aspartate aminotransferase (AST) and alanine aminotransferase (ALT).

CONTRIBUTORS

SFL, AFSR, and SRLR carried out the conduced experiments with rats. FML, APCA and LFM carried out the evaluation of diets. JAF carried out the amino acids compositions of diets. ACB and EMC contributed in the interpretation of the data. RVV and MQL conceived, designed and interpreted this study. SFL drafted the manuscript along with the other authors. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

The authors have no conflict of interest.

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TABLES

Table 1. Composition of control, low-protein and *okara* diets

Ingredients (g/kg)	**Control (17% protein)**	**Low-protein (6% protein)**	**O*kara** (17% protein)**
Casein (≥ 85% protein)	202.0	71.5	-
Okara (≥ 35% protein)	-	-	505.4
Cornstarch	397.0	480.0	279.8
Dextrinized cornstarch	130.5	159.0	93.3
Sucrose	100.0	121.0	71.0
Soybean oil	70.0	70.0	-
Fiber	50.0	50.0	-
Mineral mix (AIN-93)**	35.0	35.0	35.0
Vitamin mix (AIN-93)**	10.0	10.0	10.0
L-cystine	3.0	1.0	3.0
Choline bitartrate	2.5	2.5	2.5

**Okara* composition (%): protein 36, fat 19, carbohydrate 36, total fiber 32, humidity 6, and ash 3. **See Reeves et al., 1993.

Table 2. Amino acids composition of diets

Amino acids	Diet		
	Control (%)	***Okara* (%)**	**Low protein (%)**
Aspartic acid	1.18	1.52	0.31
Glutamine acid	3.57	3.22	1.13
Serine	0.92	0.98	0.30
Glycine	0.32	0.84	0.11
Histidine	0.42	0.33	0.12
Arginine	0.6	1.48	0.21
Threonine	0.71	0.72	0.20
Alanine	0.56	0.92	0.19
Proline	1.66	0.99	0.56
Tyrosine	0.77	0.69	0.24
Valine	1.02	0.96	0.36
Methionine	0.62	0.35	0.16
Cystine	0.27	0.18	0.04
Isoleucine	0.83	0.91	0.28
Leucine	1.57	1.53	0.54
Phenylalanine	0.79	0.99	0.28
Lysine	1.2	1.14	0.41
Total	17.01	17.70	5.42

Table 3. Isoflavone composition of dry *okara*

Isoflavone	**(%)**
Daidzin	10.1 (0.0)[a]
Malonyl daidzin	7.9 (0.1)[a]
Acetyl daidzin	ND<0.3[b]
Daidzein	3.7 (0.4)[a]
Glycitin	6.2 (0.1)[a]
Malonyl glycitin	3.8 (0.0)[a]
Acetyl glycitin	1.1 (0.1)[a]
Glycitein	1.6 (0.4)[a]
Genistin	27.1 (0.5)[a]
Malonyl genistin	25.7 (0.0)[a]
Acetyl genistin	3.1 (0.2)[a]
Genistein	10.2 (0.6)[a]
Coumestrol	UD<0.3[b]
Sissotrin	UD<0.3[b]
Biochanin A	UD<0.3[b]

[a]mean and estimation of standard deviation
[b]undetected

Table 4. Food efficiency ratio (FER), protein efficiency ratio (PER), net protein ratio (NPR) and true digestibility (TD) of casein and *okara* diets

Variables	**Diets**	
	Casein (6)	***Okara* (6)**
FER	0.34 ± 0.07	0.62 ± 0.09*
PER	3.42 ± 0.74	2.75 ± 0.28
NPR	3.74 ± 0.68	3.12 ± 0.26
TD	0.91 ± 0.01	0.92 ± 0.02

Values represent the mean ± standard deviation for the number of rats in parentheses. *Means significantly different by unpaired *t* tests ($p<0.05$).

Table 5. Final body weight, weight variation, total and relative energy intake obtained from adult rats maintained on a control (CC and LC groups), *okara* (CO and LO groups), or low-protein (LL group) diet after weaning

Variables	Groups				
	CC	**CO**	**LC**	**LO**	**LL**
Final body weight (g)	356±49 (23)	366±38 (25)	288±32^{*a} (22)	299±14^{*a} (18)	116±28^{b} (22)
Weight gain (g)	308±45 (23)	330±355$^{\#}$ (25)	259±28^{*a} (22)	274±14$^{*\#a}$ (18)	86±26^{b} (22)
Total energy intake (kcal)	4373±567^{A} (15)	3068±282^{B} (25)	3033±327^{Ba} (22)	2650±60^{Cb} (18)	1429±101^{c} (22)
(kcal/100g WB)	1263±87 (15)	960±129$^{\#}$ (25)	1184±125^{*b} (22)	929±59$^{*\#c}$ (18)	1649±270^{a} (22)

Values represent the mean ± standard deviation for the number of rats in parentheses. Means with different superscript uppercase letters are significantly different by two-way ANOVA, and those with superscript lowercase letters are significantly different by one-way ANOVA followed by LSD test ($p<0.05$). * indicates the difference between nutritional states, and # indicates the difference between diets.

Table 6. Absolute and relative weight of organs obtained from adult rats maintained on a control (CC and LC groups), *okara* (CO and LO groups), or low-protein (LL group) diet after weaning

Variables	Groups				
	CC	**CO**	**LC**	**LO**	**LL**
Liver (g)	15.8±2.9 (28)	14.6±2.2 (34)	12.3±1.9^{*a} (31)	12.0±1.5^{*a} (38)	7.8±1.6^{b} (36)
(%)	4.1±0.4^{A} (28)	3.6±0.4^{C} (34)	3.9±0.5^{ABb} (31)	3.7±0.4^{BCb} (38)	5.4±1.4^{a} (37)
Muscle (g)	0.9±0.1 (17)	1.2±0.2 (20)	0.8±0.1^{*a} (17)	0.8±0.1^{*a} (21)	0.4±0.1^{b} (17)
(%)	0.27±0.03 (17)	0.26±0.04 (20)	0.27±0.04 (17)	0.26±0.02 (21)	0.29±0.08 (17)
Epididymal fat (g)	4.3±2.4^{B} (17)	6.7±1.8^{A} (20)	3.3±0.9^{Ba} (17)	3.4±1.2^{Ba} (21)	1.9±0.7^{b} (17)
(%)	1.2±0.5^{B} (17)	1.7±0.3^{A} (20)	1.0±0.3^{Bb} (17)	1.1±0.3^{Bb} (21)	1.3±0.2^{a} (17)
Retroperitoneal fat (g)	4.7±2.5^{B} (17)	7.7±2.1^{A} (20)	4.4±1.4^{Ba} (17)	4.3±1.5^{Ba} (21)	2.1±0.9^{b} (17)
(%)	1.3±0.5^{B} (17)	1.9±0.3^{A} (20)	1.4±0.4^{B} (17)	1.3±0.4^{B} (21)	1.4±0.3 (17)

Values represent the mean ± standard deviation for the number of rats in parentheses. Means with different superscript uppercase letters are significantly different by two-way ANOVA, and those with superscript lowercase letters are significantly different by one-way ANOVA followed by LSD test ($p<0.05$). * indicates the difference between nutritional states.

Table 7. Serum urea, total protein, albumin, globulin, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) concentrations, and lipid profile obtained from adult rats maintained on a control (CC and LC groups), *okara* (CO and LO groups), or low-protein (LL group) diet after weaning

Variáveis	**Groups**				
	CC	**CO**	**LC**	**LO**	**LL**
Urea (mg/dL)	24.0±6.7 (05)	32.5±5.5$^{\#}$ (06)	30.8±4.1^{*b} (05)	42.2±6.5$^{*\#a}$ (06)	23.3±5.7^{b} (03)
Creatinine (mg/dL)	0.4±0.07 (05)	0.4±0.0 (06)	0.4±0.0^{b} (05)	0.4±0.05^{a} (06)	0.5±0.0^{a} (04)
Total protein (g/dL)	6.3±0.6 (05)	6.8±0.3 (06)	7.0±0.3^{*a} (05)	7.1±0.3^{*a} (06)	6.1±0.3^{b} (04)
Albumin (g/dL)	3.8±0.08^{C} (05)	4.3±0.18^{B} (06)	4.4±0.13^{ABa} (05)	4.6±0.19^{Aa} (06)	3.9±0.22^{b} (04)
Globulin (g/dL)	2.5±0.5 (05)	2.5±0.2 (06)	2.7±0.2^{a} (05)	2.5±0.2^{ab} (06)	2.3±0.2^{b} (04)
AST (U/L)	80±14 (05)	98±29 (04)	97±34 (04)	103±33 (06)	94±14 (03)
ALT (U/L)	39±4 (05)	44±10 (04)	46±6 (04)	48±9 (06)	35±6 (03)
Triglycerides (mg/dL)	50±7 (04)	33±6 (06)	49±28 (05)	40±16 (06)	41±8 (04)
Total cholesterol (mg/dL)	93±20^{AB} (05)	92±18^{AB} (06)	114±18^{Aa} (05)	75±16^{Bb} (05)	123±42^{a} (04)
HDL (mg/dL)	29±5^{AB} (05)	27±5^{BC} (06)	34±5^{Aa} (05)	23±3^{Cb} (06)	31±7^{a} (04)
LDL (mg/dL)	62±5 (04)	59±12$^{\#}$ (06)	69±16^{a} (05)	45±13$^{\#b}$ (06)	84±34^{a} (04)
VLDL (mg/dL)	14±9 (05)	7±1 (06)	10±5 (05)	8±3 (06)	8±1 (04)

Values represent the mean ± standard deviation for the number of rats in parentheses. Means with different superscript uppercase letters are significantly different by two-way ANOVA, and those with superscript lowercase letters are significantly different by one-way ANOVA followed by LSD test ($p<0.05$). * indicates the difference between nutritional states, and # indicates the difference between diets.

Table 8. Serum glucose, insulin and glucagon concentrations as well as insulin:glucose and glucagon:insulin ratios from adult rats maintained on a control (CC and LC groups), *okara* (CO and LO groups), or low-protein (LL group) diet after weaning

Variables	Groups				
	CC	**CO**	**LC**	**LO**	**LL**
Fasting state					
Glucose (mmol/L)	7.0±0.7 (05)	6.3±0.5 (06)	6.5±0.6 (04)	6.1±0.4 (06)	6.3±0.3 (05)
Insulin (pmol/L)	347±260 (05)	279±123 (06)	199±52^{a} (05)	148±39^{ab} (06)	119±41^{b} (05)
Insulin/glucose ratio	76±55 (05)	67±25 (06)	50±7^{a} (04)	37±8^{b} (06)	29±9^{b} (05)
Glucagon (pmol/L)	44±10 (04)	47±8 (05)	46±12 (06)	36±4 (05)	34±12 (05)
Glucagon/insulin ratio (pmol/L)	0.26±0.07 (04)	0.23±0.11 (05)	0.24±0.07 (05)	0.24±0.07 (05)	0.30±0.10 (05)
Fed state					
Glucose (mmol/L)	8.0±0.3^{A} (04)	6.3±0.4^{B} (06)	6.7±0.6^{B} (06)	6.6±0.5^{B} (08)	6.9±0.7 (06)
Insulin (pmol/L)	893±130^{A} (04)	276±109^{B} (06)	344±180^{Ba} (06)	316±123^{Ba} (08)	140±57^{b} (06)
Insulin/glucose ratio	174±24^{A} (04)	68±25^{B} (06)	80±45^{Ba} (06)	76±32^{Ba} (08)	32±13^{b} (06)
Glucagon (pmol/L)	27±17 (04)	34±10 (06)	42±18 (06)	39±14 (08)	29±8 (05)
Glucagon/insulin ratio (pmol/L)	0.03±0.02^{B} (04)	0.14±0.06^{A} (06)	0.13±0.05^{Ab} (06)	0.13±0.05^{Ab} (08)	0.22±0.09^{a} (05)

Values represent the mean ± standard deviation for the number of rats in parentheses. Means with different superscript uppercase letters are significantly different by two-way ANOVA, and those with superscript lowercase letters are significantly different by one-way ANOVA followed by LSD test ($p<0.05$).

Table 9. Glycogen liver, fat liver and glycogen muscle from adult rats maintained on a control (CC and LC groups), *okara* (CO and LO groups), or low-protein (LL group) diet after weaning

Variables	Groups				
	CC	**CO**	**LC**	**LO**	**LL**
Fasting state					
Glycogen liver (mg/100mg)	0.12±0.08 (03)	0.23±0.13 (05)	0.13±0.03 (04)	0.15±0.04 (05)	0.20±0.02 (03)
Fat liver (mg/g)	57±6 (05)	$70±12^{\#}$ (06)	$54±7^{b}$ (04)	$64±7^{\#b}$ (06)	$81±19^{a}$ (04)
Glycogen muscle (mg/100mg)	0.05±0.010 (05)	0.06±0.009 (06)	0.05±0.009 (05)	0.05±0.008 (06)	0.04±0.008 (04)
Fed state					
Glycogen liver (mg/100mg)	6.4±2.6 (06)	$2.7±1.0^{\#}$ (06)	$6.1±2.5^{a}$ (06)	$3.2±1.1^{\#b}$ (05)	$7.4±2.2^{a}$ (06)
Fat liver (mg/g)	42±5 (06)	$55±9^{\#}$ (06)	$46±6^{b}$ (05)	$59±7^{\#ab}$ (04)	$64±14^{a}$ (06)
Glycogen muscle (mg/100mg)	$0.06±0.015$ (04)	$0.04±0.011^{\#}$ (06)	$0.05±0.008^{b}$ (04)	$0.05±0.005^{\#b}$ (05)	$0.09±0.042^{a}$ (05)

Values represent the mean ± standard deviation for the number of rats in parentheses. Means with different superscript uppercase letters are significantly different by two-way ANOVA, and those with superscript lowercase letters are significantly different by one-way ANOVA followed by LSD test ($p<0.05$). # indicates the difference between diets.

FIGURES

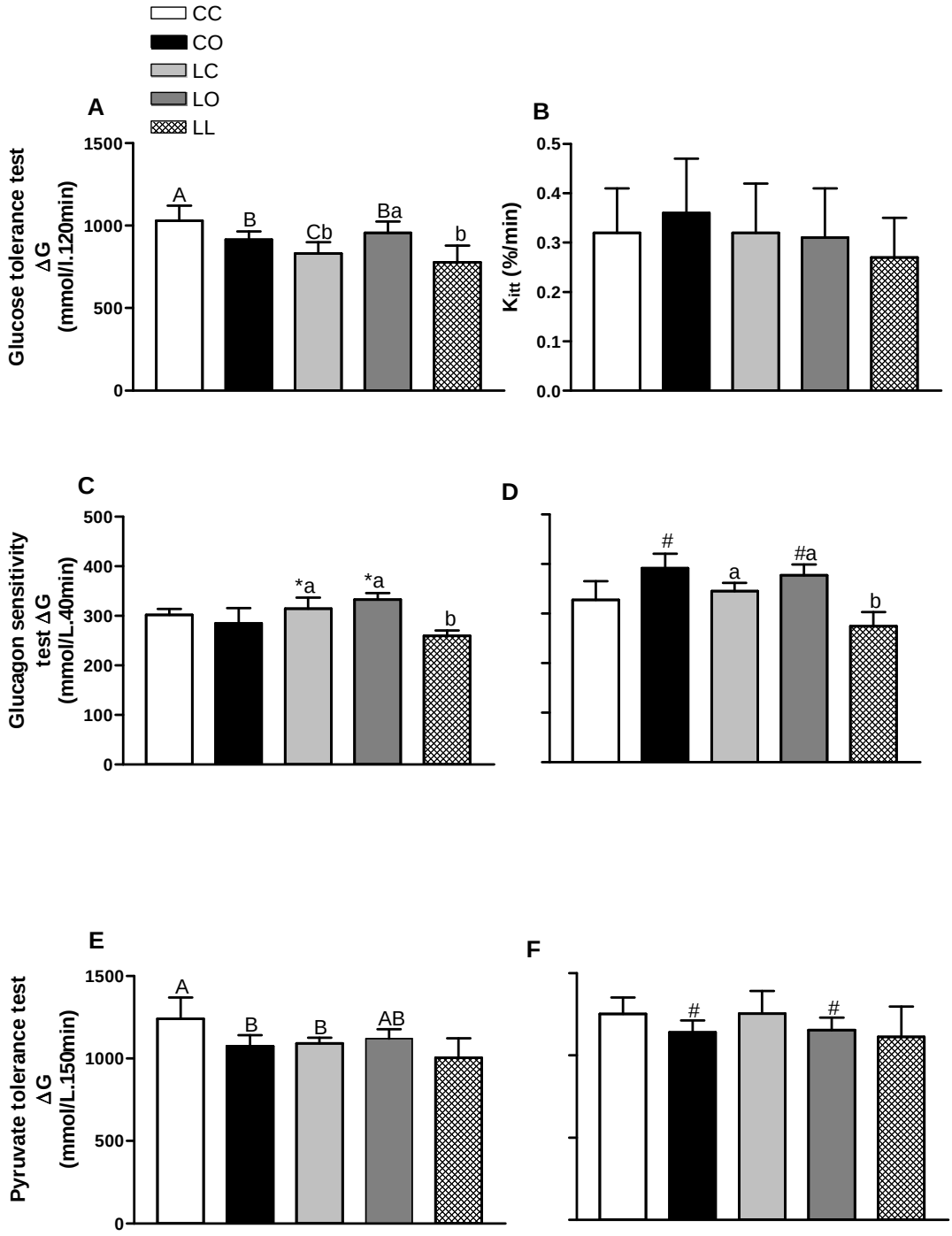

Figure 1. The mean total areas under the curves during the glucose tolerance test (A). The rate constant for serum glucose disappearance (K_{itt}) during the insulin tolerance test (B). The mean total areas under the curves during the glucagon sensitivity test after 6 h of fasting (C) and in the fed state (D). The mean total areas under the curves during pyruvate tolerance test after 12 h of fasting (E) and in the fed state (F) performed on adult rats maintained on a control (CC and LC groups), *okara* (CO and LO groups), or low-protein (LL group) diet after weaning. The bars represent the mean ± standard deviation. Mean values within a column with uppercase superscript letters are significantly different by two-way ANOVA and with lowercase superscript letters are significantly different by one-way ANOVA

followed by LSD test ($p<0.05$). * indicates the difference between nutritional states, and # indicates difference between diets.