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

Efeitos da dieta à base de *okara* sobre o intestino e o gasto energético de ratos submetidos à restrição proteica na vida intrauterina e lactação e recuperados após o desmame

Faena Moura de Lima

Cuiabá-MT
Maio/2013

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

Efeitos da dieta à base de *okara* sobre o intestino e o gasto energético de ratos submetidos à restrição proteica na vida intrauterina e lactação e recuperados após o desmame

Dissertação de Mestrado apresentada ao Programa de Pós-graduação em Biociências da Universidade Federal de Mato Grosso para a obtenção do título de Mestre.

Área de concentração: Nutrição

Linha de pesquisa: Metabolismo e transdução de sinais

Aluna: Faena Moura de Lima

Orientador: Prof. Dr. Roberto Vilela Veloso

Co-Orientadora: Profa. Dra. Márcia Queiroz Latorraca

Cuiabá-MT

Maio/2013

Dados Internacionais de Catalogação na Fonte.

L732e Lima, Faena Moura de.
Efeitos da dieta à base de okara sobre o intestino e o gasto energético de ratos submetidos à restrição proteica na vida intrauterina e lactação e recuperados após o desmame / Faena Moura de Lima. -- 2013
xv, 55 f. : il. color. ; 30 cm.

Orientador: Roberto Vilela Veloso.
Co-orientadora: Márcia Queiroz Latorraca.
Dissertação (mestrado) - Universidade Federal de Mato Grosso, Faculdade de Nutrição, Programa de Pós-Graduação em Biociências, Cuiabá, 2013.
Inclui bibliografia.

1. tolerância ao frio. 2. recuperação nutricional. 3. okara. 4.

Ficha catalográfica elaborada automaticamente de acordo com os dados fornecidos pelo(a) autor(a).

BANCA EXAMINADORA

Prof. Dr. Roberto Vilela Veloso

Assinatura

Prof.ª Dr.ª Valéria Ernestânia Chaves

Assinatura

Prof.ª Dr.ª Maria Salete Ferreira Martins

Assinatura

Prof.ª Dr.ª Maria Helena Gaíva Gomes da Silva

Assinatura

Cuiabá, 02 de maio de 2013.

“Hoje me sinto mais forte, mais feliz quem sabe, só levo a certeza de que muito pouco eu sei ou nada sei...
Cada um de nós compõe a sua história, cada ser em si carrega o dom de ser capaz e ser feliz...”

Renato Teixeira e Almir Sater

AGRADECIMENTOS

A **Deus**, pela dádiva da vida, do aprendizado, por todas as bênçãos recebidas que me fazem seguir mais forte e por todos os anjos que colocou em meu caminho.

A minha **família**, especialmente aos meus amados pais, **Leontina** e **Osvaldo**, pelo incentivo que sempre me deram, até nas mais difíceis decisões. Pelos exemplos de amor, caráter, perseverança, entre tantos outros.

Ao meu namorado, **Helder Borges**, que há mais de 6 anos me acompanha em todos os momentos, sempre me encorajando quando nem eu mesma acreditava que daria certo. Obrigada pelo amor, paciência, confiança, companheirismo e dedicação. Vencemos mais essa fase juntos!

Aos meus eternos amigos, **André, Jaline, Ludmila, Suzana, Talitta, Thaís e agregados**, que compartilham comigo a jornada desde a graduação. Vocês foram meu alicerce, minha família, meu socorro e minhas vitórias. Com vocês o fardo ficou mais leve.

Ao meu orientador, **Roberto Vilela Veloso**, pela oportunidade concedida desde a época da graduação, pela confiança, contribuições e por todos os momentos que me alegrou com sua presença, sempre educado e bem humorado. Um exemplo de cordialidade.

À co-orientadora, **Márcia Queiroz Latorraca**, pela atenção despendida ao trabalho e ensinamentos.

Aos demais professores do LABA, **Maria Helena, Maria Salete, Marise e Vanessa,** pelos conhecimentos compartilhados.

Ao querido **Celso**, que sempre muito atencioso não mede esforços em nos ajudar. Obrigada pelos conselhos, você é essencial para as pesquisas realizadas, exemplo de dedicação ao trabalho.

À querida **Sílvia**, por sempre estar disposta a auxiliar e compartilhar seus conhecimentos. Aprendi muito com você.

Às amigas especiais, **Ana Paula e Nelma,** foi um grande presente conhecer vocês, obrigada por sempre estarem dispostas, pela preocupação e por todos os ensinamentos compartilhados, não só no mestrado mais também fora dele.

À **Kariny e Lunara**, que me acrescentaram muito, pessoal e profissionalmente. Exemplos de dedicação e responsabilidade, sempre interessadas e ativas. Obrigada pela amizade, por todos os momentos e a força que me deram. Já sinto saudades.

Às grandes amigas que fiz no mestrado, **Amanda, Clarissa, Fernanda, Marina, Bruna e Hellen.** Tenho muito orgulho de vocês, cada uma com sua qualidade me acrescentou algo. Todas guerreiras, esforçadas, inteligentes, "fitness e poliglotas". Obrigada por compartilharem comigo o trem da alegria, as palhaçadas, o "bullying" e os lanches na Menina Bolinho. Jamais me esquecerei de como fui feliz na companhia de vocês, espero encontrá-las muitas vezes ainda.

Aos alunos de iniciação científica, **Katarine, Naiara, Caroline, José Rui e Ana Luisa**, pela disposição em ajudar.

Aos técnicos do LABA e funcionários da UFMT, **Jorge, Natalino, Iraci, Rubens e Rafaela**, por auxiliarem nos trabalhos desenvolvidos e pela presteza que nos atendem.

À **Letícia Ignácio, Juliana Faria e Lucas Francisco,** que sem medir esforços me auxiliaram em tudo, foram essenciais para o desenvolvimento desse trabalho. Sou eternamente grata, espero poder retribuir.

Aos professores da UNICAMP, **Lício Augusto Velloso, Everardo Magalhães Carneiro e Gabriel Anhê,** por cederem os laboratórios e equipamentos para a realização dos experimentos.

Ao Prof. Dr. **Anibal Eugenio Vercesi**, por disponibilizar o seu laboratório e seus alunos para que eu aprendesse novas técnicas.

A todos que estiveram comigo em Campinas, **Érika, Albina Ramalho, Lívia Andrade, Adriene Paiva, Andrezza Kinote, Thiago Matos, Rafael Camargo, Andressa Coope e Rodrigo**, auxiliando nos experimentos, sempre dispostos e atenciosos. Obrigada pela companhia e ensinamentos.

Ao **Laboratório de Bioquímica e ao de Investigação**, pela contribuição durante todo o desenvolvimento do trabalho, emprestando equipamentos e reagentes quando foi necessário.

À empresa **Bioclin,** pela doação dos kits utilizados neste trabalho. Foram essenciais para conclusão da pesquisa.

À **Faculdade de Nutrição da Universidade Federal de Mato Grosso**, pelo curso de pós-graduação e pelas instalações que permitiram a realização deste trabalho.

Ao **CNPq, CAPES e FAPEMAT**, pelo apoio financeiro.

Especialmente aos **animais de experimentação**, que foram fundamentais para realizarmos nosso estudo.

A **todos** que contribuíram, direta ou indiretamente, para a realização deste trabalho.

SUMÁRIO

LISTA DE ABREVIATURAS

ADRB3	Receptor beta 3 adrenérgico
AGCC	Ácido graxo de cadeia curta
AGL	Ácidos graxos livres
AIN	American Institute of Nutrition
AMPc	Adenosina monofosfato cíclico
AOAC	Association of Official Analytical Chemists
ATGL	Lipase dos triglicerídeos específica dos adipócitos
ATP	Adenosina trifosfato
BW	Body weight
C	Dieta controle
CC	Grupo controle caseína
cDNA	DNA complementar
CO	Grupo controle *okara*
DAG	Diacilglicerol
DM2	Diabetes mellitus tipo 2
EWAT	Epididymal white adipose tissue
GAPDH	Gliceraldeído-3-fosfato desidrogenase
GTT	Teste de tolerância à glicose
HTs	Hormônios tireoidianos
IBAT	Interscapular brown adipose tissue
IBGE	Instituto Brasileiro de Geografia e Estatística
LHS	Lipase hormônio sensível
LC	Grupo recuperado caseína
LL	Grupo hipoproteico
LO	Grupo recuperado *okara*
LP	Dieta hipoproteica
LPL	Lipase lipoproteica
MAG	Monoacilglicerol
mRNA	RNA mensageiro
PCR	Reação em cadeia da polimerase

PGC-1α	Co-ativador 1 alfa do receptor ativado por proliferador de peroxissomo
RNA	Ácido ribonucleico
RWAT	Retroperitoneal white adipose tissue
SBCAL	Sociedade Brasileira de Ciência em Animais de Laboratório
SDS-PAGE	Eletroforese em gel de poliacrilamida com duodecil sulfato de sódio
T3	Triiodotironina
T4	Tiroxina
TAB	Tecido adiposo branco
TAM	Tecido adiposo marrom
TAG	Triacilglicerol
TNF-α	Fator de necrose tumoral alfa
TSH	Hormônio estimulante da tireoide
TTBS	Solução salina tamponada com Trisma base e Tween
UCP1	Proteína descacopladora 1
VO_2	Consumo de oxigênio

LISTA DE TABELAS

LISTA DE FIGURAS

RESUMO

Introdução: O *okara* é um subproduto da produção do "leite de soja" e estudos que o utilizaram como suplemento, demonstraram resultados positivos em relação ao peso corporal, esteatose hepática, metabolismo lipídico, controle glicêmico e redução do risco de complicações do diabetes, além do potencial efeito prebiótico. Em trabalhos anteriores demonstramos que esta fonte proteica possui eficiência de recuperação nutricional similar a dieta à base de caseína, no entanto, o tratamento com dieta *okara* reduziu o consumo alimentar e o peso corporal final, além de alterar a consistência e o volume fecal. **Objetivo**: Assim, a fim de elucidar se o menor peso corporal final poderia ser justificado apenas pela redução da ingestão alimentar e para verificar os efeitos intestinais do consumo de *okara*, avaliamos fatores associados ao gasto energético e ao intestino nesse modelo animal. **Métodos:** Foram avaliadas crias de ratas que consumiram dieta normoproteica (17% de proteína) ou hipoproteica (6% de proteína) à base de caseína durante a prenhez e a lactação e que foram mantidas com dieta com 17% de caseína (CC e LC), 17% de *okara* (CO e LO) ou 6% de caseína (LL) do desmame a vida adulta. Aos 60 dias de vida foi realizado o teste de tolerância ao frio por 5h e atividade espontânea em 24h; e aos 90 dias o consumo de oxigênio. Após eutanásia aos 90 dias de vida, amostras de tecido adiposo branco epididimal e retroperitoneal foram coletadas para determinação da lipólise *ex vivo* e do conteúdo proteico da enzima lipase hormônio sensível (LHS) apenas no epididimal. O tecido adiposo marrom interescapular (TAMI) foi coletado para determinação da expressão gênica do receptor beta 3 adrenérgico (ADRB3), do co-ativador 1 alfa do receptor ativado por proliferador de peroxissomo (PGC-1α), da proteína desacopladora 1 (UCP1), da lipase dos triglicerídeos específica dos adipócitos (ATGL) e da LHS, além do conteúdo proteico de LHS, ATGL, ADRB3 e UCP1. O intestino foi coletado e pesado, determinado o comprimento, a umidade fecal e o pH cecal. O sangue foi coletado para determinação das concentrações séricas de triiodotironina (T3), tiroxina (T4) e hormônio estimulante da tireoide (TSH). **Resultados:** A recuperação nutricional, independente da dieta, diminuiu os níveis de T3 e a hiperfagia, aumentou o peso e os níveis de TSH comparado aos animais LL. Os animais CO e LO comparados com CC e LC, apresentaram menor consumo alimentar total, de carboidratos, ganho de peso, consumo de oxigênio (VO_2) e pH cecal, porém maior consumo de lipídeos, peso intestinal, umidade fecal, expressão de mRNA de PGC1α e UCP1 no TAMI,

termogênese estimulada pelo frio (com 3h de exposição) e taxa de lipólise *ex vivo* estimulada no tecido adiposo epididimal. **Conclusão:** Em conclusão, o subproduto *okara* foi eficiente no controle de peso e redução de depósitos de gordura em animais recuperados da desnutrição, mesmo aparentemente diminuindo fatores associados ao gasto energético (VO_2 e atividade espontânea), além de possuir efeitos intestinais benéficos que independem do estado nutricional.

Palavras-chave: tolerância ao frio, recuperação nutricional, okara, tecido adiposo, ratos.

INTRODUÇÃO

Nas últimas décadas, o Brasil e os demais países em desenvolvimento passaram por alterações na estrutura da dieta que inverteram o padrão de distribuição dos problemas nutricionais (Sartorelli & Franco, 2003; Schramm et al., 2004). Entre 1974 e 2009 ocorreu uma redução intensa e contínua da desnutrição em ambos os sexos na população infantil e adulta brasileira, medida através da prevalência de déficit de altura e deficit de peso (IBGE, 2010). Apesar disso, a prevalência do deficit de altura ainda é quase duas vezes maior que a prevalência de deficit de peso em crianças de 5 a 9 anos de idade (IBGE, 2010) e embora estejam reduzidos podem estar relacionados com a insuficiência alimentar, tanto em quantidade com em qualidade (Sawaya et al., 2003).

No mesmo período, o excesso de peso quase triplicou entre homens, já a obesidade cresceu cerca de quatro vezes entre os homens e cerca de duas vezes entre as mulheres (IBGE, 2010) e assim como a insuficiência alimentar (Sawaya et al., 2003), o aumento da obesidade pode estar muitas vezes associado ao aumento da morbidade. Como visto por Schramm et al. (2004), as doenças crônico-degenerativas respondem por 66,3% da carga de doença no Brasil e essa predominância da morbidade gera um impacto importante na qualidade de vida do indivíduo e nos gastos públicos que serve como alerta da necessidade em conhecer as causas e as consequências desses tipos de agravos.

Após inúmeros estudos epidemiológicos e experimentais, acredita-se que entre as causas da obesidade e de doenças crônicas, há uma combinação da genética com fatores ambientais, como dieta, sedentarismo e desenvolvimento intra-uterino (Sartorelli & Franco, 2003).

Entre as hipóteses que relacionam os fatores ambientais envolvidos no desenvolvimento de doenças, temos a hipótese do fenótipo econômico, que surgiu há mais de vinte anos numa tentativa de explicar as associações entre o crescimento fetal e infantil deficiente e um maior risco de desenvolver intolerância à glicose e a síndrome metabólica na vida adulta (Hales & Barker, 2001). A programação de um organismo multicelular envolve uma série de eventos sincronizados, impulsionados por instruções genéticas adquiridas durante fases críticas do desenvolvimento, como a concepção e a lactação (Patel & Srinivasan, 2002). No caso da desnutrição, a mãe mal nutrida irá transmitir essencialmente ao feto uma previsão do ambiente nutricional em que vai

nascer e como resposta à desnutrição fetal sucedem adaptações metabólicas vantajosas para a sobrevivência pós-natal, como a preservação seletiva de órgãos-chave (Hales & Barker, 2001). É uma manifestação do fenômeno da "plasticidade" do desenvolvimento e isso é vantajoso em termos evolutivos, pois permite que um genótipo, em resposta a variadas condições ambientais durante o desenvolvimento, dê origem a diferentes fenótipos e estados fisiológicos ou morfológicos (Barker, 2007). Essas adaptações ocorrem de maneira permanente, mesmo cessado o estímulo nutricional responsável por essas alterações e continuam a ser expressas a nível celular, molecular e bioquímico, tornando-se prejudiciais quando o ambiente pós-natal difere do ambiente intra-uterino (Patel & Srinivasan, 2002).

Em animais experimentais, existem grandes diferenças no impacto da nutrição materna deficiente sobre o crescimento dos diferentes órgãos da prole. Isso acontece pela diferença na sensibilidade de cada órgão em responder aos efeitos da subnutrição, podendo afetar principalmente as células de divisão rápida, como o trato gastrointestinal, limitando o seu crescimento e função (Weaver et al., 1998).

Foi observado em filhotes de mães com restrição alimentar apenas durante a gestação, que o peso e o comprimento do intestino delgado foram estatisticamente menores ao nascimento e também após a lactação, assim como a altura total da mucosa, a altura da vilosidade e a do enterócito (Gurmini et al., 2005). Em modelo experimental de restrição proteica, o comprimento intestinal foi analisado no 21º dia de vida e os autores concluíram que o mais afetado foi o grupo de filhotes de mães com restrição proteica durante a gestação e a lactação, seguido pelo grupo de filhotes de mães com restrição protéica somente na lactação, sugerindo que a restrição protéica pré-natal isolada não apresenta efeitos negativos duradouros sobre as dimensões e o crescimento do trato gastrintestinal (Weaver et al., 1998).

Segundo Latorraca et al. (1998), a recuperação nutricional com dieta balanceada do nascimento ou desmame até a vida adulta não é capaz de reverter integralmente as alterações da programação metabólica, especialmente o déficit de secreção de insulina que é compensado pelo aumento da sensibilidade periférica a esse hormônio.

Estudos mostram que crianças desnutridas que não recuperaram a estatura e consomem uma dieta rica em gorduras, tem maior susceptibilidade para acumular gordura corporal (Sawaya et al., 1998; Sawaya et al., 2003). O acúmulo de gordura corporal depende, dentre outros fatores, do desequilíbrio no balanço energético entre a

energia ingerida e a energia gasta e cerca de 40% desse balanço depende da herança genética (Marques-Lopes et al., 2004).

O tecido responsável por armazenar esse excesso de energia na forma de triacilglicerol (TAG) é o tecido adiposo branco (TAB). As células dos adipócitos brancos são esféricas e com tamanho variável dependendo da quantidade de TAG armazenados em sua única gotícula lipídica, que é responsável por mais de 90% do volume da célula (Saely et al., 2012). As principais atividades metabólicas desse tecido, além do seu papel como órgão endócrino, envolvem a lipogênese e a lipólise (Fonseca-Alaniz et al., 2006).

A lipogênese envolve os processos metabólicos em que o excesso de energia resulta em biossíntese, incorporação e armazenamento de TAG na gotícula de gordura intracitoplasmática (Fonseca-Alaniz et al., 2006). A lipólise é o processo de mobilização de TAG com liberação de ácido graxo livre (AGL) e glicerol, distribuídos através da circulação para os tecidos periféricos, podendo ser utilizados para β-oxidação e a produção de ATP, por exemplo (Lass et al., 2011). Esse processo ocorre durante o jejum ou em condições de grande necessidade de energia, como o exercício físico e situações de estresse e é estimulado principalmente por catecolaminas (Fonseca-Alaniz et al., 2006). As duas enzimas hidrolases mais importantes nesse processo são: a lipase hormônio sensível (LHS), que hidrolisa uma variedade de substratos, como TAG, monoacilglicerol (MAG), mas principalmente diacilglicerol (DAG) e a desnutrina (ou ATGL), que é uma hidrolase seletiva para TAG. As duas juntas são responsáveis por mais de 90% da capacidade lipolítica no tecido adiposo de ratos (Lass et al., 2011).

O gasto de energia, em homeotérmicos, é o resultado da atividade física, do metabolismo basal e termogênese facultativa (Puigserver & Spiegelman, 2003) e representa a conversão de oxigênio e alimento (ou os estoques de energia) em dióxido de carbono, água, calor e trabalho (Lowell & Spiegelman, 2000). A atividade física refere-se a todo movimento voluntário (Spiegelman & Flier, 2001) e no caso do rato, essa atividade é maior no ciclo escuro do que no claro (Richter, 1922). O metabolismo basal envolve os inúmeros processos bioquímicos necessários para a sobrevivência (Spiegelman & Flier, 2001) e ele não é linear mesmo quando comparado com animais da mesma espécie, por ser influenciado além de outros fatores, pela idade, sub ou superalimentação, massa corporal total, altura e pela taxa metabólica específica de cada órgão (Even et al., 2001). A termogênese facultativa compreende a mudança na

dissipação de calor em consequência a alterações de temperatura ambiental, estado nutricional ou infecção. Em pequenos mamíferos essa termogênese adaptativa ocorre principalmente no tecido adiposo marrom (TAM), sendo estimulada principalmente pela ação dos hormônios tireoidianos (HTs) e ativação adrenérgica (Puigserver & Spiegelman, 2003).

O tecido adiposo marrom é composto por adipócitos que contém TAG em numerosos pequenos vacúolos, sendo que as organelas mais características são as mitocôndrias e a cor desse tecido é atribuída pela alta densidade dessa organela e pela alta vascularização (Saely et al., 2012). As mitocôndrias do TAM são marcadas pela expressão da proteína desacopladora 1 (UCP1), que desacopla a síntese de ATP a partir da fosforilação oxidativa na cadeia respiratória, resultando na produção de calor (Cinti, 2012).

Em mamíferos, os HTs atuam em diversas vias metabólicas, como na regulação da temperatura corporal, no consumo de oxigênio, metabolismo de carboidratos, proteínas e gorduras, além de exercerem efeito no desenvolvimento de vários tecidos (Barra et al., 2004). Os hormônios triiodotironina (T3) e tiroxina (T4) são sintetizados na glândula tireoide e o hormônio estimulante da tireoide (TSH) é o principal regulador de secreção dos HTs (Yen, 2001). O papel do HT na estimulação da biogênese mitocondrial ocorre através do núcleo ativada por T3. Alguns dos genes nucleares influenciados por esse hormônio, como PGC-1α, parecem regular a expressão do genoma mitocondrial e outros podem participar como componentes funcionais na cadeia respiratória, como o citocromo c1 (Nelson, 1990).

A exposição ao frio e alimentação (hipoproteica ou hiperlipídica, por exemplo) aumenta a atividade do TAM e dependendo da duração e intensidade do estímulo, pode aumentar a expressão da UCP1 através da norepinefrina liberada pelo sistema nervoso simpático, que se liga ao receptor β-adrenérgico, aumentando as concentrações intracelulares de adenosina monofosfato cíclico (AMPc). Ratos expostos ao frio e células de TAM tratadas com agonista do receptor β-adrenérgico (ADRB3) apresentam forte indução da transcrição de mRNA do co-ativador-1 'alfa' do receptor ativado por proliferador do peroxissoma (PGC-1α), o qual induz e coordena a expressão de genes que estimulam o metabolismo oxidativo mitocondrial no TAM (Puigserver & Spiegelman, 2003).

Todo esse processo complexo que envolve o equilíbrio do balanço energético demonstra que a terapia de sucesso contra obesidade deve impactar não só o consumo alimentar, mas também o gasto de energia ou até ambos. Quando essa terapia é baseada na orientação nutricional, pode ocorrer uma perda de peso favorável para a redução de consequências mórbidas (Spiegelman & Flier, 2001) e quanto mais precoces forem as intervenções maior a possibilidade de sucesso nas mudanças de estilo de vida (Sartorelli & Franco, 2003).

Em relação à terapia nutricional auxiliar na obesidade ou desnutrição, inclui-se a soja, que além de ser uma fonte proteica de alta qualidade (Silva et al., 2006), de vitaminas do complexo B e minerais, como ferro, potássio e magnésio (Erdman & Fordyce, 1989; Aplevicz & Demiate, 2007), fornece inúmeras substâncias bioativas benéficas à saúde e por essa razão é considerada um alimento funcional (Morais & Silva, 2000; Casé et al., 2005; Penha et al., 2007). O Brasil é o segundo maior produtor mundial de soja (Pelaez et al., 2004) e Mato Grosso é o maior produtor nacional com uma área plantada de 6,4 milhões de hectares (CONAB, 2012). No mundo, apenas 7% da produção de soja destina-se para alimentação humana e os subprodutos obtidos pelo processamento dos grãos são geralmente descartados ou utilizados para fabricação de ração animal como farelo ou concentrado proteico (Amaral, 2006; Bellaver & Snizek, 2011).

Em um estudo realizado por Cederroth et al. (2008), os animais que foram submetidos à uma dieta à base de soja (com alto teor de fitoestrógeno) apresentaram redução da adiposidade corporal e melhora da sensibilidade à insulina. Além disso, recentemente, foi observado que a recuperação da desnutrição proteica em fases críticas do desenvolvimento com dieta à base de farinha integral de soja é capaz de restaurar parcialmente o déficit de secreção de insulina (Veloso et al., 2008), elevando as concentrações séricas de insulina nos estados basal e pós-prandial e apresentando maior área sob a curva de insulina em resposta a uma carga de glicose (GTT intraperitoneal) (Oliveira et al., 2008).

A partir do processamento da soja, diferentes produtos e subprodutos são originados e entre eles podemos citar o *tofu*, *tempe* e o *okara*, que é um resíduo insolúvel vegetal considerado de baixo custo (Aplevicz & Demiate, 2007), obtido a partir da lavagem, maceração e aquecimento dos grãos de soja para produção do extrato aquoso de soja (“leite de soja”) (Nakornpanom et al., 2010) e corresponde a cerca de

25% da produção deste alimento (Perusselo, 2008). O *okara* possui elevado valor nutritivo, contém em torno de 25% de proteína (Yokomizo et al., 2002), 10 a 15% de gordura (O'Toole, 1999), 42% de fibras, 4 a 6% de carboidratos e 2,8% cinzas (Bowles e Demiate, 2006), além de vitaminas e minerais. No entanto, frequentemente o *okara* é utilizado como resíduo industrial, sendo descartado de forma incorreta no meio ambiente ou empregado na fabricação de ração animal (Katayama & Wilson, 2008).

Sabe-se também, que o *okara* possui de 12 a 14% de fibras solúveis (Nakornpanom et al., 2010) e que esses componentes da dieta não são absorvidos pelo organismo, porém ao passarem pelo intestino grosso são utilizados como substrato pelas bactérias intestinais através da fermentação. As bactérias responsáveis por esse processo são as anaeróbicas localizadas no cólon e como produto desta reação temos o ácido lático, ácidos graxos de cadeia curta (AGCC) e gases, com conseqüente redução do pH do lúmen e proliferação das células epiteliais desta região (Saad, 2006; Venema, 2010).

Diferentes mecanismos foram propostos para tentar explicar a correlação entre o intestino e o controle do peso corporal, entre eles, incluem o papel da flora intestinal em extrair energia de polissacarídeos não digeríveis e em regular genes do hospedeiro que modulam a utilização e o armazenamento de energia (Tsukumo et al., 2009). Matsuo (1996) ao suplementar uma dieta de 20% de proteína de soja com 50% de *okara* (OC) ou 50% de *okara koji* (OK) (*okara* fermentado), observou maior ingestão e utilização de fibras dietéticas no grupo OC, porém menor pH cecal, absorção de ferro, ácidos biliares e colesterol fecal. Jiménez-Escrig et al. (2008), encontraram maior absorção aparente e retenção verdadeira de cálcio em ratos alimentados com dieta de *okara* rica em fibras (DFRO), assim como o status antioxidante, peso e umidade fecal. Além disso, a suplementação com 10% de *okara* a uma dieta padrão resultou em maior peso cecal e conteúdo total de AGCC e menor pH em ratas *Wistar*, demonstrando que o *okara* pode ser eficaz como um suplemento dietético para perda de peso pelo seu potencial efeito prebiótico (Préstamo et al., 2007)

Em modelo experimental de obesidade induzida, o crescente aumento da dose de *okara* (10, 20 ou 40%) na dieta, suprimiu o desenvolvimento do ganho de peso e de tecido adiposo branco epididimal, preveniu o aumento de lipídeos plasmáticos, incluindo colesterol total, LDL colesterol e ácidos graxos livres. Além disso, a ingestão de *okara* preveniu a esteatose hepática e suprimiu a expressão dos genes da leptina e TNFα (Matsumoto, 2007). Ahmed et al. (2010), ao induzirem diabetes em machos

Sprague Dawley (DM) e suplementá-los com *okara* (DM – *okara*), verificaram reduzidos níveis de glicose, hemoglobina glicosilada e aumento dos níveis de insulina, além disso a suplementação melhorou as alterações histopatológicas observadas no pâncreas quando comparados aos animais DM. Desse modo, por seu baixo custo, elevado valor nutricional e propriedades funcionais, o *okara* poderia ser um alimento alternativo para a recuperação nutricional de modelo animal desnutrido em fases críticas do desenvolvimento, que é propenso a desenvolver DM2 e obesidade na vida adulta.

Utilizando uma dieta com 17% de proteína à base de *okara* na recuperação de um modelo animal desnutrido na gestação e lactação, demonstramos através de parâmetros bioquímicos e hormonais que esta fonte proteica possui eficiência de recuperação nutricional similar a dieta à base de caseína (Lemes SF, Lima FM, Carli APA, Ramalho AFS, Reis SRL, Michelotto LF, Amaya-Farfán J, Carneiro EM, Boschero AC, Latorraca MQ and Veloso RV, dados não publicados). No entanto, o tratamento com *okara,* independente do estado nutricional, reduziu o consumo alimentar e o peso corporal final, além de alterar a consistência e o volume fecal. Assim, a fim de elucidar se o menor peso corporal final poderia ser justificado apenas pela redução da ingestão alimentar e para verificar os efeitos intestinais do consumo de *okara*, avaliamos fatores associados ao gasto energético e ao intestino nesse modelo animal.

REFERÊNCIAS BIBLIOGRÁFICAS

Ahmed LA, Hassan DR, Hemeda HM. Anti-hyperglycemic effects of okara, corn hull and their combination in alloxan induced diabetic rats. World App Sci J 2010; 9(10): 1139–47.

Amaral VMG. A importância da soja como alimento funcional para a qualidade de vida e saúde [dissertação]. Campinas: UNICAMP; 2006. 86 p.

Aplevicz KS & Demiate IM. Análises físico-químicas de pré-misturas de pães de queijo e produção de pães de queijo com adição de *okara.* Ciênc agrotec 2007; 31 (5): 1416-22.

Barra GB, Velasco LFR, Pessanha RP, Campos AM, Moura FN, Dias SMG, Polikarpov I, Ribeiro RCJ, Simeoni LA, Neves FAR. Mecanismo molecular da ação do hormônio tireoideano. Arq Bras Endocrinol Metab 2004; 48(1): 25-39.

Barker DJP. The origins of the developmental origins theory. J Intern Med 2007; 261(5): 412–17.

Bellaver C & Snizek PN. Processamento da soja e suas implicações na alimentação de suínos e aves. Concórdia; 1998 [Acesso em 07 agosto 2011]. Disponível em: http://www.cnpsa.embrapa.br/sgc/sgc_arquivos/palestras_g0r65h6e.pdf.

Bowles S & Demiate IM. Caracterização físico-química de *okara* e aplicação em pães do tipo francês. Ciênc Tecnol Aliment 2006; 26(3): 652-59.

Casé F, Deliza R, Rosenthal A, Mantovani D, Felberg I. Produção de 'leite' de soja enriquecido com cálcio. Ciênc Tecnol Aliment 2005; 25(1): 86-91.

Cederroth CR, Vinciguerra M, Gjinovci A, Kühne F, Klein M, Cederroth M, *et. al*. Dietary phytoestrogens activate amp-activated protein kinase with improvement in lipid and glucose metabolism. Diabetes 2008; 57(5):1176–85.

Cinti S. The adipose organ at a glance. Dis Model Mech 2012; 5(5): 588-94.

Companhia Nacional de Abastecimento. Acompanhamento da safra brasileira de grãos 2010/2011 – Décimo Levantamento - Maio/2012. [Acesso em 07 junho 2012]. Disponível em:

http://www.conab.gov.br/OlalaCMS/uploads/arquivos/12_05_21_11_00_43_ia-mai12.pdf.

Erdman JWJ & Fordyce EJ. Soy products and the human diet. Am J Clin Nutr l989; 49(5): 725-37.

Even PC, Rolland V, Roseau S, Bouthegourd JC, Tomé D. Prediction of basal metabolism from organ size in the rat: relationship to strain, feeding, age and obesity. Am J Physiol Regul Integr Comp Physiol 2001; 280(6): 1887-96.

Fonseca-Alaniz MH, Takada J, Alonso-Vale MIC, Lima FB. O tecido adiposo como centro regulador do metabolismo. Arq Bras Endocrinol Metab 2006; 50(2): 216-29.

Gurmini J, Cecílio WAC, Schuler SL, Olandoski M, Noronha L. Desnutrição intra-uterina e suas alterações no intestino delgado de ratos Wistar ao nascimento e após a lactação. J Bras Patol Med Lab 2005; 41(4): 271-8.

Hales CN & Barker DJP. The thrifty phenotype hypothesis. Br Med Bull 2001; 60(1): 5-20.

Instituto Brasileiro de Geografia e Estatística. Pesquisa de Orçamentos Familiares 2008-2009: Antropometria e estado nutricional de crianças, adolescentes e adultos no Brasil. 2010.

Jiménez-Escrig A, Tenorio MD, Espinosa-Martos I, Rupérez P. Health-promoting effects of a dietary fiber concentrate from the soybean byproduct okara in rats. J Agric Food Chem 2008; 56(16): 7495–501.

Katayama M & Wilson LA. Utilization of okara, a byproduct from soymilk production, through the development of soy-based snack food. J Food Sci 2008; 73(3): 152-57.

Lass A, Zimmermann R, Oberer M, Zechner R. Lipolysis – A highly regulated multi-enzyme complex mediates the catabolism of cellular fat stores. Prog Lipid Res 2011; 50(1): 14–27.

Latorraca MQ, Reis MAB, Carneiro EM, Mello MAR, Velloso LA, Saad MJA, *et al*. Protein deficiency and nutritional recovery modulate insulin secretion and the early steps of insulin action in rats. J Nutr 1998; 128(10): 1643-49.

Lowell BB & Spiegelman BM. Towards a molecular understanding of adaptive thermogenesis. Nature 2000; 404(6778): 652-60.

Marques-Lopes I, Marti A, Moreno-Aliaga MJ, Martínez A. Aspectos genéticos da obesidade. Rev Nutr 2004; 17(3): 327-38.

Matsumoto K, Watanabe Y, Yokoyama S. Okara, soybean residue, prevents obesity in a diet induced murine obesity model. Biosc Biotech Bioch 2007; 71(3): 720-27.

Matsuo M. Acceleration of sterol excretion, little meteorism and less inhibition of iron absorption by *okara koji*, a new foodstuff, in rats. Biosc Biotech Bioch 1996; 60 (4): 571-74.

Morais AAC & Silva AL. Valor nutritivo e funcional da soja. Rev Bras Nutr Clín 2000; 15(2): 306-15.

Nakornpanom NN, Hongsprabhas P, Hongsprabhas P. Effect of soy residue (okara) on in vitro protein digestibility and oil release in high-calorie emulsion stabilized by heated mixed proteins. Food Research International 2010; 43(1): 26–32.

Nelson BD. Thyroid hormone regulation of mitochondrial function: Comments on the mechanism of signal transduction. Biochim Biophys Acta 1990; 1018(2-3): 275-77.

O'Toole DK. Characteristics and use of okara, the soybean residue from soy milk productions: a review. J Agric Food Chem 1999; 47(2): 363-71.

Oliveira EA, Cheim LMG, Veloso RV, Arantes VC, Reis MAB, Carneiro EM, et al. Nutritional recovery with a soybean flour diet improves the insulin response to a glucose load without modifying glucose homeostasis. Nutrition 2008; 24(1): 76-83.

Patel MS & Srinivasan M. Metabolic programming: causes and consequences. J Biol Chem 2002; 277(3): 1629-32.

Pelaez V, Albergoni L, Guerra MP. Soja transgênica versus soja convencional: uma análise comparativa de custos e benefícios. Cadernos de Ciência e Tecnologia 2004; 21(2): 279-309.

Penha LAO, Fonseca ICB, Mandarino JM, Benassi VT. A soja como alimento: valor nutricional, benefícios para a saúde e cultivo orgânico. Boletim do Centro de Pesquisa de Processamento de Alimentos 2007; 25(1): 91-102.

Perusselo CA. Estudo dos parâmetros de processo e modelagem numérica da secagem do resíduo sólido na produção do extrato hidrossolúvel de soja (okara) [dissertação]. Curitiba: Pontifícia Universidade Católica do Paraná; 2008. 110p.

Préstamo G, Rupérez P, Espinosa-Martos I, Villanueva MJ, Lasunción MA. The effects of okara on rat growth, cecal fermentation, and serum lipids. Eur Food Res Technol 2007; 225(5-6): 925–28.

Puigserver P & Spiegelman BM. Peroxisome proliferator-activated receptor-γ coactivator 1α (pgc-1α): transcriptional coactivator and metabolic regulator. Endocr Rev 2003; 24(1): 78–90.

Richter CP. A behavioristic study of the activity of the rat. Comp Psychol Monogr 1922; 1(2): 56p.

Saad SMI. Probióticos e prebióticos: o estado da arte. Rev Bras Cienc Farm 2006; 42(1): 1-16.

Saely CH, Geiger K, Drexel H. Brown versus white adipose tissue: a mini-review. Gerontology 2012; 58(1): 15–23.

Sartorelli DS & Franco LJ. Tendências do diabetes mellitus no Brasil: o papel da transição nutricional. Cad Saúde Pública 2003: 19 Suppl 1: 29-36.

Sawaya AL, Solymos GMB, Florêncio TMMT, Martins PA. Os dois Brasis: quem são, onde estão e como vivem os pobres brasileiros. Estud av 2003; 17(48): 21-44.

Sawaya AL, Grillo LP, Verreschi I, Silva AC, Roberts SB. "Mild stunting is associated with higher susceptibility to the effects of high fat diets: studies in a shantytown population in São Paulo, Brazil". J Nutr 1998; 128 Suppl 2: 415-20.

Schramm JMA, Oliveira AF, Leite IC, Valente JG, Gadelha AMJ, Portela MC, *et al*. Transição epidemiológica e o estudo de carga de doença no Brasil. Ciência e Saúde Coletiva 2004; 9(4): 897-908.

Silva MS, Naves MMV, Oliveira RBO, Leite OSM. Composição química e valor protéico do resíduo de soja em relação ao grão de soja**.** Ciênc Tecnol Aliment 2006; 26(3): 571-76.

Spiegelman BM & Flier JS. Obesity and the regulation of energy balance. Cell 2001; 104(4): 531–43.

Tsukumo DM, Carvalho BM, Carvalho-Filho MA, Saad MJA. Translational research into gut microbiota: new horizons in obesity treatment. Arq Bras Endocrinol Metab 2009; 53(2): 139-44.

Veloso RV, Latorraca MQ, Arantes VC, Reis MAB, Ferreira F, Boschero AC, Carneiro EM. Soybean diet improves insulin secretion through activation of cAMP/PKA pathway in rats. J Nutr Bioch 2008;19(11): 778-84.

Venema K. Role of gut microbiota in the control of energy and carbohydrate metabolism. Curr Opin Clin Nutr Metab Care 2010; 13(4): 432–38.

Villanueva AMJ, Yokoyama BWH, Hong BYJ, Barttley BGE, Rupérez CP. Effect of high-fat diets supplemented with okara soybean by-product on lipid profiles of plasma, liver and faeces in Syrian hamsters. Food Chemistry 2011; 124(1): 72–79.

Weaver LT, Desai M, Austin S, Arthur HML, Lucas A, Hales NC. Effects of protein restriction in early life on growth and function of the gastrointestinal tract of the rat. J Pediatr Gastroenterol Nutr 1998; 27(5): 553-9.

Yen PM. Physiological and molecular basis of thyroid hormone action. Physiol Rev 2001; 81(3): 1097-142.

Yokomizo A, Takenaka Y, Takenaka T. Antioxidative activity of peptides prepared from okara protein. Food Sci Technol Res 2002; 8(4): 357–59.

OBJETIVOS

Objetivo geral

Avaliar os efeitos da dieta à base de *okara* sobre o intestino e o gasto energético em ratos adultos submetidos à restrição proteica na vida intrauterina e na lactação e recuperados após o desmame.

Objetivos específicos

Analisar o perfil nutricional e metabólico de ratos adultos, determinando:

- o consumo alimentar (total e de macronutrientes);
- o peso corporal;
- o peso de órgãos (intestino e tecidos adiposos epididimal, retroperitoneal e marrom interescapular);
- o consumo de oxigênio e a atividade física espontânea;
- produção de calor durante o teste de tolerância ao frio;
- liberação de glicerol após o teste de lipólise *ex vivo*.

Determinar o perfil sérico hormonal tireoidiano de ratos adultos, dosando:

- T3;
- T4;
- TSH.

Avaliar o efeito do *okara* sobre o intestino, determinando:

- comprimento e peso intestinal (absoluto e relativo);
- pH cecal;
- umidade fecal.

Verificar no tecido adiposo marrom interescapular a expressão do mRNA:

- LHS;
- UCP1;
- PGC-1α.

Verificar no tecido adiposo marrom interescapular o conteúdo proteico:

- ADRβ3;
- ATGl;
- LHS;
- UCP1.

Verificar no tecido adiposo branco epididimal estimulado o conteúdo proteico:

- LHS total;
- LHS fosforilada.

ARTIGO

Okara diet increases cold tolerance and attenuates body fat accumulation in rats recovered from protein restriction in early life

Faena Moura de Lima[1], Kariny Cássia de Siqueira[1], Ana Paula Carli de Almeida[1], Lucas Francisco Ribeiro do Nascimento[2], Sílvia Regina de Lima Reis[3], Márcia Queiroz Latorraca[3], Roberto Vilela Veloso[3].

[1]Mestrado em Biociências, Faculdade de Nutrição, Universidade Federal de Mato Grosso, Cuiabá, MT, Brazil.

[2]Laboratório de Sinalização Celular, Universidade Estadual de Campinas, Campinas, São Paulo, Brazil.

[3]Laboratório de Avaliação Biológica de Alimentos, Departamento de Alimentos Nutrição, Faculdade de Nutrição, Universidade Federal de Mato Grosso, Cuiabá, MT, Brazil.

Corresponding author: Faena Moura de Lima, Mestrado em Biociências, Faculdade de Nutrição, Universidade Federal de Mato Grosso, Avenida Fernando Correa da Costa, 2367. Bairro Boa Esperança. Cuiabá, MT, Brazil - 78060-900 Phone: +55-65-3615 8814 Fax: +55-65-3615 8811. E-mail: faena5@hotmail.com

Figures: 6

Tables: 5

Running title: *Okara* diet increases cold tolerance

Key words: cold tolerance, nutritional recovery, *okara*, adipose tissue, rats

ABSTRACT

In previous work we demonstrated by biochemical and hormonal parameters that the *okara* as protein source possesses efficiency of nutritional recovery similar to diet based on casein, however, treatment with diet okara, independent of nutritional status, reduced food intake and the final body weight in addition to changing the volume and fecal consistence. Thus, in order to elucidate whether the lower final body weight could be justified only by the reduction of food intake and to verify the intestinal effects of the consumption of okara, we evaluated factors associated with energy expenditure and the intestine in this animal model. Only male rats from mothers that were fed either 17% or 6% protein during pregnancy and lactation were maintained on a 17% casein (CC and LC), 17% *okara* (CO and LO) or 6% casein (LL) diet after weaning until adult age. The nutritional recovery, independent of protein source, reduced the levels of triiodothyronine (T3), however increased the levels of thyroid stimulating hormone (TSH), decreased hyperphagia and increased weight observed in animals under protein restriction. Rats fed with *okara* based diet exhibited reduced the total food intake, smaller weight gain and also the cecum presented low values of pH compared to animals fed with casein. These animals showed higher lipids intake, the mRNA expression of PGC1α and UCP1 in interscapular brown adipose tissue, the adaptive thermogenesis and the *ex vivo* lipolysis rate stimulated on epididymal adipose tissue. In conclusion, we have demonstrated that the subproduct *okara* is efficient on weight control and reduction of fat deposits on animals recovered from malnutrition, even without increasing the energy expenditure, besides promoting beneficial intestinal effects despite the nutritional status.

INTRODUCTION

Over the past few decades, Brazil and other developing countries have been lived diet structural changes that inverted the nutritional issues, previously characterized by malnutrition and now tagged by increase the obesity and consequently non transmitted chronic diseases (Sartorelli & Franco, 2003; Schramm et al., 2004). Epidemiological and experimental studies have been demonstrating that malnutrition on critical phases of development, as conception and lactation (Hales & Barker, 2001), is able to generate advantageous physiological adaptations through genetic instructions. These modifications allow the survival in similar nutritional situations and this become harmful when the postnatal environment become different from the information transmitted by mother, as an overnutrion and consequently obesity (Patel & Srinivasan, 2002). Those metabolic adaptations are permanent and even when the nutritional recovery is done by balanced diet from birth to weaning until adult life, it is not capable of fully revert the metabolic programming changes (Latorraca et al., 1998).

As a result, the animals can present metabolic diseases like obesity and associated disorders. For this reason, many substances have been investigated as aids in decreasing the fat deposition and weight control (Chen et al., 2012). The manufacturing "soy milk" subproduct, *okara*, has high nutritional value, besides vitamins and minerals contains approximately 25% of protein (Yokomizo, Takenaka & Takenaka, 2002), 10 to 15% of fat (O'Toole, 1999), 4 to 6% of carbohydrates and 2.8 % of ashes (Bowles & Demiate, 2006). Still possess 40.2-43.6% of insoluble fiber and 12.6-14.6% of soluble fiber (Nakornpanom, Hongsprabhas & Hongsprabhas, 2010), distributed mostly among cellulose, hemicelluloses and lignin (O'Toole, 1999). Despite its high nutritional value, frequently the *okara* is used as an industrial residue, being inappropriately discarded on the environment or used in the manufacturing of animal food (Katayama & Wilson, 2008). Studies with *okara* as an supplement, showed positive results related to body weight and hepatic steatosis (Matsumoto, Watanabe & Yokoyama, 2007; Jiménez-Escrig et al., 2008), lipid metabolism (Villanueva *et al*., 2011), glycemic control and reduction of the risk of diabetes complications (Ahmed, Hassan & Hemeda, 2010), in addition to its potential prebiotic effect (Matsuo, 1996; Préstamo *et al*., 2007). In summary, considering its low cost, elevated nutritional value and functional properties, the *okara* could be an alternative food to the nutritional recovery of a malnourished

animal model on critical phases of development, which is prone to develop type 2 diabetes and obesity on adult life.

Utilizing a diet with 17% protein okara based in the recovery animal model of malnutrition during gestation and lactation, we demonstrate through biochemical and hormonal parameters that this protein source possesses efficiency of recovery nutritional similar diet based on casein (unpublished data). However, treatment with okara independent of nutritional status, reduced food intake and final body weight in addition to changing the volume and fecal consistency. Thus, in order to elucidate whether the lower final body weight could be justified only by the reduction of food intake and whether there influence of intestinal by the consumption of okara, we evaluated factors associated with energy expenditure and the intestine on adult rats submitted to intrauterine and lactation protein restriction and recovered 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 and maintained until 90 days of age 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 centesimal composition of o*kara* was analyzed using established methods. The compositions of the 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 *et al*., 1993). The diets fatty acids composition was determined by prior acid hydrolysis (method A) (Zenebon & Pascuet, 2005). Nitrogen content was determined by micro-Kjeldahl method, using a nitrogen conversion factor of 6.25 (AOAC, 1995). Fiber content was determined by an enzyme-gravimetric method according to Prosky *et al*. (1984).

The rats were kept in collective cages, given free access to food and water and kept under standard lighting conditions (12 h light–12 h dark cycle) at 24°C throughout the experimental period. The rats were weighed once per week throughout the experimental period. Food intake was recorded three times per week, and the data are expressed in absolute and relative values. To assess the relative food intake, the total food intake during the experimental period after weaning was normalized per 100g body weight at age 90d.

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	140.0[#]
Fiber	50.0	50.0	161.7[#]
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
Kcal/g	3.72	3.79	3.69

**Okara* composition (%): protein 35, fat 29, carbohydrate 4, total fiber 32. **See Reeves *et al*., 1993. [#]Contained in the okara.

Oxygen consumption determination

Oxygen consumption was measured in fed animals through a computer-controlled, open circuit calorimeter system LE405 Gas Analyzer (Panlab – Harvard Apparatus, Holliston, MA, USA). Rats were singly housed in clear respiratory chambers and room air was passed through chambers at a flow rate of 0.8 L/min, maintained by a pump Air Supply & Switching and monitored by a gas analyzer LE405 (Panlab – Harvard Apparatus). Gas sensors were calibrated prior to the onset of experiments with primary gas standards containing known concentrations of O_2 and CO_2 (Air Liquid, Sao Paulo, Brazil). The analyses were performed by 24 hours of each chamber. Outdoor air reference values were sampled after every four measurements. Sample air was sequentially passed through O_2 sensors for determination of O_2 content, from which measures of oxygen consumption (VO_2) were estimated.

Evaluation of spontaneous locomotor activity

At 60 days of age, the spontaneous locomotor activity was evaluated during 24 hours by a platform contain two horizontal axes, providing information on motility

using a linear detection system controlled by computer from Harvard Instruments (Panlab, Holliston, MA, USA).

Cold tolerance test

The cold tolerance test was performed in rats at 60 days of age. Rectal temperature was measured using Physitemp TCAT-2LV thermometers before and during a 5h exposure to 4°C.

Hormonal profile and tissue collection

Rats were killed by decapitatation. Blood samples were collected, serum was obtained by centrifugation and aliquots were used to measure serum triiodothyronine (T3), thyroxine (T4) and thyroid-stimulating hormone (TSH). Concentrations of these hormones were determined by using the AccuBind ELISA Microwells (T4 and T3) and BIOLISA TSH – K100 (Bioclin) according to the manufacturer's instructions. After medial laparotomy, epididymal and retroperitoneal white adipose tissue (EWAT and RWAT) and brown adipose tissue (BAT) was quickly removed, weighed and aliquots were frozen immediately in liquid nitrogen and stored at -80°C to several determinations.

pH and fecal moisture

After medial laparotomy, entire intestine with fecal contents was removed and weighed immediately reveal (intestinal weight) then feces were removed from the rectal portion and its wet weight was obtained in electronic analytical balance (Acculab model ALC-210.4) with sensitivity of 0.0001 g. Then, drying was performed in faeces oven at 105ºC. After 22 hours, began to weighing with 30 minute intervals until two consecutive weightings been obtained with a difference less than 1.0 mg. The moisture content of the stool was calculated using the formula [(weight fecal wet - dry fecal weight) / wet fecal weight] x 100. A portion of the cecal content was diluted 1:3 in water immediately after sampling; the pH was measured using a pHmeter (DLA-PH, Del Lab) (Jiménez-Escrig et al., 2008).

Ex vivo lipolysis

To measure *ex vivo* lipolysis, the method of Chen et al. (2012) was used with modifications. Briefly, two 0.25g samples in duplicate of epididymal and retroperitoneal fat dissected from rats were minced separately and incubated for 1h in 1 mL of buffer alone (basal), containing Krebs–Ringer, 3% fatty acid-free bovine serum albumin, 6mM-glucose (Sigma-Aldrich), and pH 7.4. Then, the buffer was exchanged for basal or containing isoproterenol at a concentration of 10^{-5}M (stimulated), incubated for 1h in a water bath at 37°C and the glycerol released into the medium was measured using a triglyceride kit without lipase (Bioclin).

RNA preparation and real-time RT-PCR

Total RNA was separated from the frozen adipose tissue by Trizol reagent (Invitrogen, USA) according to the supplier's instruction. Three micrograms of total RNA was transcribed into cDNA with reverse transcriptase high capacity (Applied Biosystems). Primers specific for rat PGC1α (Rn01453110_m1), Lipe (Rn00563444_m1), UCP1 (Rn00562126_m1), Adrb3 (Rn01478698_g1), Pnpla2 (Rn01479969_m1) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) primers were obtained from Applied Biosystems. GAPDH were used as endogenous control. PCR was carried out in duplicate on a Step One using Taqman Gene Expression Master Mix (Applied Biosystems). The cDNA was amplified under the following conditions: 95°C for 10 min for denaturation and subjected to 40 cycles of 95°C for 15s, 60°C for 20 s, and 72°C for 15 s, followed extension at 72°C for 10 min. Real-time data were analyzed using the Step One System (Applied Biosystems). Quantitative values were obtained from the threshold cycle value (Ct), which is the point where a significant increase of fluorescence is first detected. Results were calculated according to $2^{-\Delta\Delta CT}$ method.

Western Blot

A fraction of IBAT and EWAT from each experimental group was homogenized by polytron in an anti-protease cocktail. After homogenization, an aliquot of extract was collected and the total protein content was determined by the dye-binding protein assay kit (Bio-Rad Laboratories, Hercules, CA). Samples of crude membrane preparations from each experimental group were incubated for 5 min at 100°C with 5X concentrated

Laemmli sample buffer (1 mmol/L sodium phosphate, pH 7.8, 0.1% bromophenol blue, 50% glycerol, 10% SDS, 2% mercaptoethanol) (4:1, v/v). The samples with 80µg protein were then run on 10% polyacrylamide gels. Electrotransfer of proteins to nitrocellulose membranes (Bio-Rad) was done for 2:30 h at 120 V (constant) in buffer containing methanol and SDS. After checking the efficiency of transfer by Ponceau S staining, the membranes were blocked with 50 g/L dry skimmed milk in TTBS (10 mmol/L Tris, 150 mmol/L NaCl, 0.5% Tween 20) overnight at 4°C. ATGL, p-HSL, HSL, UCP1, ADRB3 and GAPDH was detected in the membrane after a 4h incubation at room temperature with their respective antibodies (diluted 1:500 in TTBS) (Santa Cruz Biotechnology). The membranes were then incubated with secondary antibody (diluted 1:5000 in TTBS) followed by a further 2h incubation at room temperature. Protein bound to the antibody was detected by autoradiography. The intensity of the bands will be evaluated by densitometry Scion Image software (ScionCorporation).

Statistical analyses

The results were expressed as mean ± standard deviation. Levene's test for homogeneity of variances was initially used to check the normality of the data before testing with parametric ANOVA. When necessary, the data were log-transformed to correct for variance in heterogeneity or non-normality (Sokal & Rohlf, 1995). Two-way ANOVA analysis of variance (effects of nutritional status and diet) was used to compare the data from the CC, CO, LC, and LO groups. The same data were analyzed by one-way ANOVA analysis of variance when assessing whether diet was effective in improving 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. $P < 0.05$ indicated statistical significance. All statistical comparisons were done using the Statistic Software package (Statsoft, Tulsa, OK, USA).

RESULTS

The okara based diet reduced around 8% the total food intake and the nutritional recovery decreased the hyperphagia observed in malnourished animals (Table 2). Independent of the nutritional status, the groups treated with the *okara* based diet exhibited lower total consumption when compared with the others treated with casein ($F_{1,47}$=27.76, p<0.0001). The groups LC and LO presented lower total food intake than the groups CC and CO ($F_{1,47}$=335.42, p<0.0001). Compared with the LL group, the biggest consumption was from the LC followed by the LO (p<0.0001).

However, when expressed per 100g of body weight, the food intake was similar among groups CC, LO and CO ($F_{1,47}$=4.92, p<0.05). The LC group presented lower consumption than CC and LO and similar to the CO. Considering only nutritional recovery, the relative consumption of LC and LO groups was 30% and 21% lower, respectively, than the LL ($F_{2,36}$=27.01, p<0.0001). Both total consumption of carbohydrates and protein had influence on the nutritional status ($F_{1,47}$=350.95, p<0.0001), in which the animals recovered from malnutrition, independent of the diet, had a lower consumption of these macronutrients than the control animals. In consequence of the lower total food intake, the LL group also ingested less quantity of total fiber and all macronutrients when compared with the LC and LO groups (p<0.001). Though the animals treated with the *okara* based diet had a lower total intake of food and carbohydrates ($F_{1,47}$=578.7, p<0.0001), the total intake of lipids (p<0.0001) and fiber (p<0.0001) were 2x and 3x, respectively, bigger on the CO and LO than CC and LC.

Table 2. Total and relative food intake, total carbohydrate, protein and lipid intake 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 (13)	**CO (13)**	**LC (13)**	**LO (13)**	**LL (13)**
Total food intake (g)	1233±87	1126±39$^{\#}$	914±59^{*a}	842±41$^{*\#b}$	480±25^{c}
(g/100g WB)	291±26^{A}	283±19^{AB}	266±31^{Bb}	296±41^{Ab}	376±46^{a}
Total energy intake (Kcal)	4585±322	4278±147$^{\#}$	3401±220^{*a}	3199±155$^{*\#b}$	1771±91^{c}
(Kcal/100g WB)	1083±96	1076±73	990±115^{*b}	1036±88^{*b}	1386±170^{a}
Total carbohydrate (g)	727±51	495±17$^{\#}$	539±35^{*a}	370±18$^{\#*b}$	341±18^{c}
Total protein (g)	209±15	203±7	155±10^{*a}	151±7^{*a}	24±1^{b}
Total lipid (g)	86±6^{C}	169±6^{A}	64±4^{Db}	126±6^{Ba}	34±2^{c}
Total fiber (g)	62±4^{C}	180±6^{A}	46±3^{Db}	135±6^{Ba}	24±1^{c}

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 analysis of variance, and those with superscript lowercase letters are significantly different by one-way analysis of variance followed by LSD test ($p<0.05$). *indicates the difference between nutritional states, and $^{\#}$indicates the difference between diets.

The treatment with the okara based diet decreased in 9% the weight gain ($p<0.001$) and independent of the diet, the nutritional recovery improved the weight gain in malnourished animals (Table 3). At the end of the experimental period, the body weight ($F_{1,47}=93.45$, $p<0.0001$) and the weight gain ($F_{1,47}=36.13$, $p<0.0001$), were lower on the LC and LO groups when compared with CC and CO. In addition, analyzing the nutritional recovery, the highest body weight ($F_{2,36}=282.65$, $p<0.0001$) and weight gain ($F_{2,36}=397.59$, $p<0.0001$) were found on the LC group, followed by LO and LL. The fat deposit on the epididymal and retroperitoneal adipose tissues was lower on the recovered animals when compared to controls ($F_{1,16}=27.75$, $p<0.0001$ and $F_{1,16}=22.07$, $p<0.001$). The LC group obtained, on both tissues, the biggest absolute weight, followed by LO and LL ($p<0.0001$). To the interscapular brown adipose tissue there was no significant difference between groups. When expressed per 100g of body weight, the weight of epididymal ($F_{1,16}=15.92$, $p<0.001$) and retroperitoneal ($F_{1,16}=9.05$, $p<0.01$)

adipose tissue were lower in the LC and LO groups when compared with the CC and CO. Also on both tissues, the relative weight was similar between the LO and LL groups and these 27% (RWAT) and 23% (EWAT) lower than LC ($p<0.05$). In brown adipose tissue there was similarity among LC and LO groups and these lower than LL ($F_{2,13}=48.02$, $p<0.0001$).

Table 3. Final body weight, weight gain, absolute and relative weight of epididymal, retoperitoneal and brown fat 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 (13)	**CO (13)**	**LC (13)**	**LO (12)**	**LL (14)**
Final body weight (g)	425±35	399±32$^{\#}$	346±32^{*a}	310±24$^{\#*b}$	130±18^{c}
Weight gain (g)	362±31	335±29$^{\#}$	318±31^{*a}	283±22$^{*\#b}$	102±15^{c}
Epididymal fat (g)	7±3	6.5±0.9	4±0.8^{*a}	3±0.4^{*b}	1.5±0.4^{c}
(g/100g WB)	1.5±0.4	1.4±0.3	1.1±0.2^{*a}	0.8±0.1^{*b}	0.9±0.1^{b}
Retroperitoneal fat (g)	8±3	7.6±0.9	5±0.1^{*a}	4±0.8^{*b}	1.8±0.7^{c}
(g/100g WB)	1.8±0.5	1.6±0.2	1.5±0.3^{*a}	1.1±0.3^{*b}	1.1±0.1^{b}
Interscapular brown fat (g)	0.5±0.08	0.6±0.1	0.5±0.1	0.4±0.04	0.5±0.1
(g/100g WB)	0.11±0.01	0.13±0.02	0.15±0.05^{b}	0.14±0.02^{b}	0.29±0.03^{a}

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 analysis of variance, and those with superscript lowercase letters are significantly different by one-way analysis of variance followed by LSD test ($p<0.05$). * indicates the difference between nutritional states, and $^{\#}$ indicates the difference between diets.

As thyroid hormones (THs) regulates several metabolic pathways, including the carbohydrates, proteins and lipids, we measured the concentration of triiodothyronine (T3), thyroxine (T4) and thyroid stimulating hormone (TSH) from the serum of these animals and we found that nutritional recovery, independent of the diet, decreased the T3 levels, however increased TSH levels (Table 4). The T3 levels were lower in LC and LO when compared with control animals ($F_{1,15}=9.75$; $p<0.01$) and with LL animals ($F_{2,12}=17.96$, $p<0.001$). When it comes to T4, there was no difference related to control

animals, but compared to LL animals, this hormone levels were smaller on the LC and LO groups ($F_{2,12}$=12.5, p<0.01). The nutritional status had influence on TSH levels and in this case, the recovery animals LC and LO exhibited higher values than control animals ($F_{1,15}$=15.20; p<0.01).

Table 4. Hormonal parameters of 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 (5)	**CO (4)**	**LC (5)**	**LO (5)**	**LL (5)**
T3 (ng/mL)	0.72±0.2	0.74±0.3	0.44±0.1^{*b}	0.47±0.2^{*b}	1.1±0.3^{a}
T4 (µg/dl)	1.5±0.4	1.8±0.6	1.7±0.3^{b}	2.3±0.4^{b}	3.2±0.6^{a}
TSH (µUI/mL)	0.35±0.02	0.37±0.01	0.40±0.02*	0.39±0.02*	0.43±0.07

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 analysis of variance, and those with superscript lowercase letters are significantly different by one-way analysis of variance followed by LSD test (p<0.05). * indicates the difference between nutritional states, and $^{\#}$ indicates the difference between diets.

Due to the high content of soluble fiber present in the based okara diet, we evaluated the intestinal effects of this treatment and regardless of nutritional status, there was modulation of the majority evaluated intestinal parameters (Table 5). The intestinal absolute weight was higher in the CO group, followed by LO, CC and LC ($F_{1,15}$=10.47, p<0.01). Between groups recovered from malnutrition, the LO had obtain higher absolute weight than LC and this was higher than LL ($F_{2,14}$=128.88, p<0.0001). When expressed per 100g of body weight, the intestinal weight was higher in animals fed with based *okara* diet ($F_{1,16}$=66.81, p<0.0001). Furthermore, the LO group had the highest relative intestinal weight, followed by LL and this was higher than LC ($F_{2,14}$=32.75, p<0.0001). The intestinal length was lower in recovery animals of malnutrition LC and LO relative to CC and CO control ($F_{1,15}$=10.07, p<0.01). When compared with the LL group, LC and LO groups were bigger and these were similar to each other ($F_{2,14}$=28.63, p<0.0001). When expressed per body length, intestinal length did not differ significantly among groups. The cecal pH was lower in LO and CO animals when compared with CC and LC and both were similar to each other ($F_{1,15}$=5.28, p<0.05). The LL group was similar to LC and both higher than LO

($F_{2,12}$=91.03, p<0.0001). The fecal moisture was 45% higher in animals treated with based *okara* diet when compared with animals treated with casein, independently of nutritional status ($F_{1,16}$=41.83, p<0.0001). The LL group exhibited fecal moisture similar to LC group and both were lower than LO ($F_{2,12}$=26.72, p<0.0001).

Table 5. Weight and length, absolute and relative of intestinal, cecal pH and fecal moisture 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 (5)	**CO (4)**	**LC (5)**	**LO (6)**	**LL (6)**
Intestinal weight (g)	16 ± 1^{C}	26 ± 3^{A}	13 ± 0.5^{Db}	19 ± 1^{Ba}	7 ± 2^{c}
(g/100g BW)	3.8±0.6	$5.9\pm0.8^{\#}$	3.8 ± 0.4^{c}	$5.7\pm0.4^{\#a}$	4.4 ± 0.4^{b}
Intestinal length (cm)	129±9	136±10	$122\pm4^{*a}$	$119\pm8^{*a}$	93 ± 8^{b}
Intestinal length relative	5.2±0.4	5.4±0.5	5.4±0.3	5.1±0.4	5.1±0.4
Cecal pH	7.5 ± 0.2^{A}	6.5 ± 0.2^{B}	7.6 ± 0.1^{Aa}	6.2 ± 0.1^{Cb}	7.6 ± 0.2^{a}
Fecal moisture (%)	52±3	$70\pm6^{\#}$	50 ± 1^{b}	$77\pm12^{\#a}$	49 ± 8^{b}

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 analysis of variance, and those with superscript lowercase letters are significantly different by one-way analysis of variance followed by LSD test (p<0.05). * indicates the difference between nutritional states, and # indicates the difference between diets.

As the animals treated with okara consume more lipids and the nutritional recovery with okara decreased the EWAT and RWAT weight, we carried out analyzing oxygen consumption to check the energy expenditure of those animals (Figure 1). The oxygen consumption was lower on animals treated with based *okara* diet (CO and LO) when compared with CC and LC animals (p<0.05). The light phase had an additional effect on nutritional status, when recovery animals LC and LO exhibited lower VO_2 than CC and CO animals. The LC animals had shown higher O_2 consumption than LO and LL animals and on the dark phase there was significant difference among the last two groups, with LL maintaining the lowest rate of VO_2.

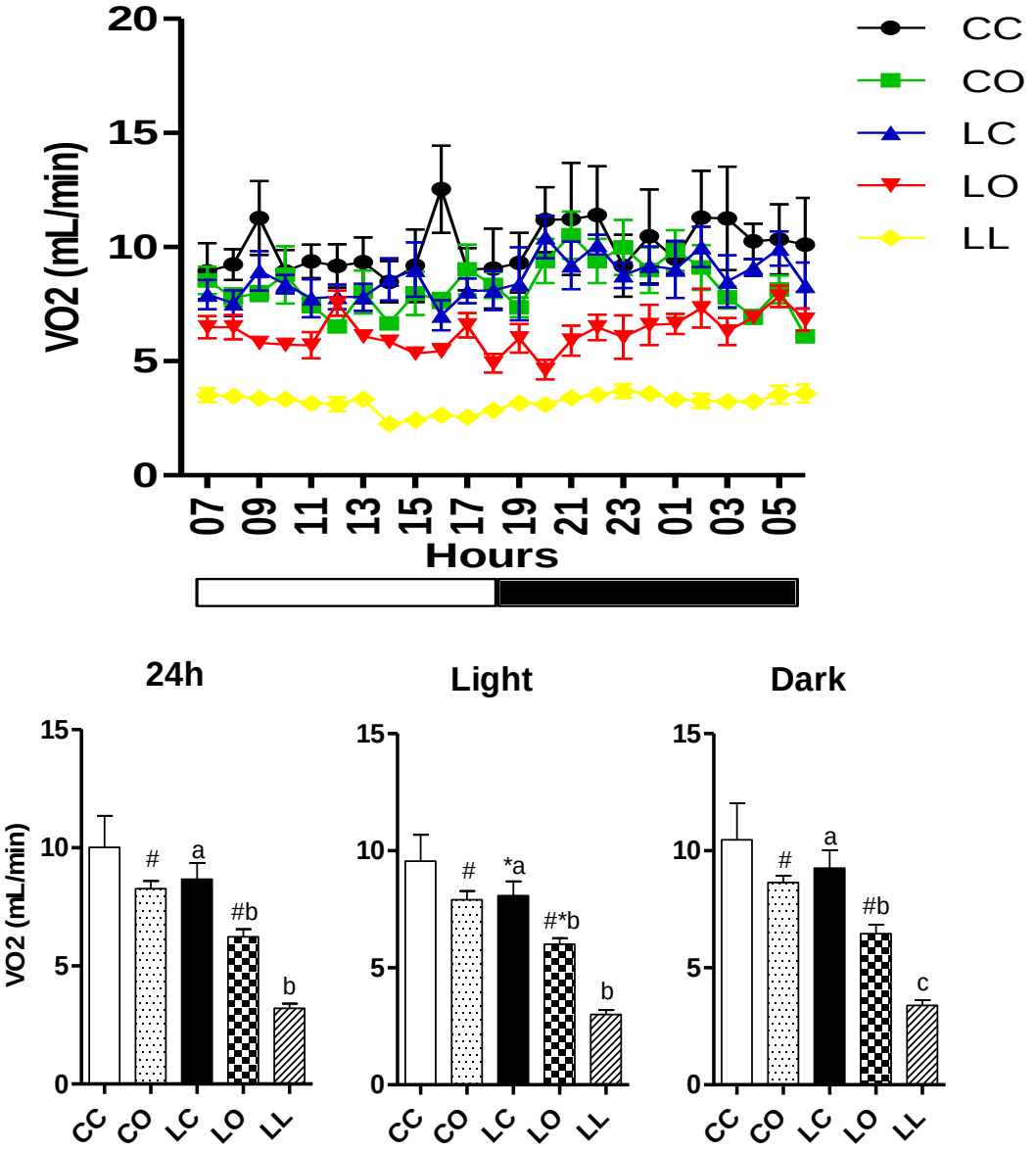

Figure 1. Oxygen consumption of 24 hours, light and dark cycle of adult rats maintained on control casein (CC and LC groups), *okara* (CO and LO groups) or low-protein (LL group) diets after weaning. The bars are expressed as the mean ± SEM (n=4 rats) of oxygen consumption measured during the entire period cited. Means with different uppercase letters are significantly different by two-way analysis of variance followed by Holm-Sidak method, and those with lowercase letters are significantly different by one-way analysis of variance followed by Tukey test ($p<0.05$). *indicates the difference between nutritional states, and $^{\#}$indicates the difference between diets.

According to the O_2 consumption, recovery animals LC and LO moved less than the control animals ($F_{1,14}=66.90$; $p<0.0001$) and more than LL group in the light phase. Moreover, in 24 h of acquisition data, LO animals presented a pattern of spontaneous activity lower than LC animals ($p<0.0001$).

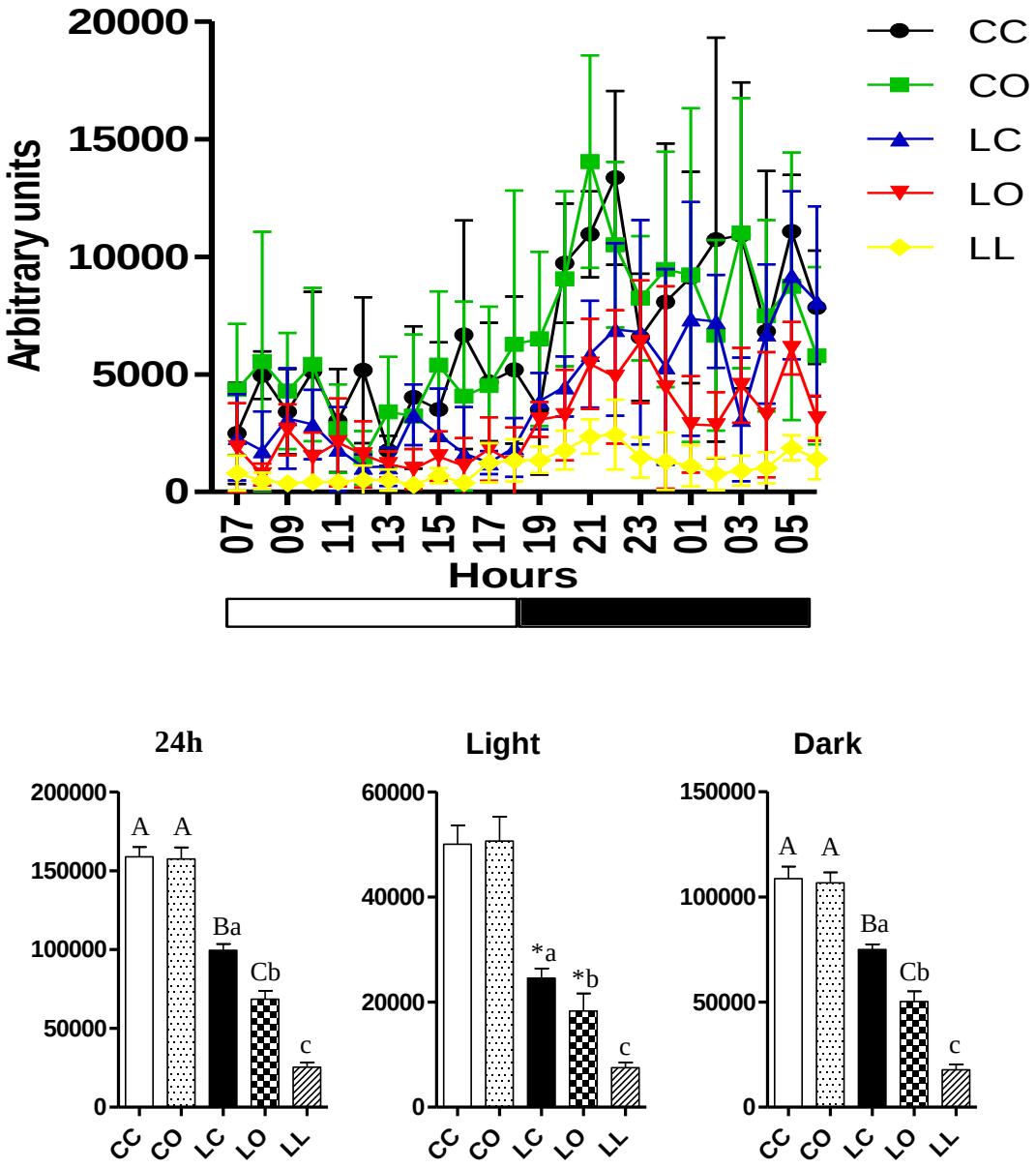

Figure 2. Spontaneous activity of 24 hours, light and dark cycle of adult rats maintained on control casein (CC and LC groups), *okara* (CO and LO groups) or low-protein (LL group) diets after weaning. The bars correspond to spontaneous activity of the period in arbitrary units and are expressed as the mean ± SEM (n= 4-5 rats). Means with different uppercase letters are significantly different by two-way analysis of variance followed by Holm-Sidak method, and those with lowercase letters are significantly different by one-way analysis of variance followed by Tukey test ($p<0.05$). *indicates the difference between nutritional states, and $^{\#}$indicates the difference between diets.

Due to the high-fat diet okara, we investigated the capacity thermogenic of those animals through the gene expression (Figure 3) *and protein content* (Figure 4) *of pro-thermogenic factors in IBAT and a test of tolerance to cold* (Figure 5). The CO and LO

groups shown higher mRNA levels to peroxisome proliferator-activated receptor-γ coactivator (PGC1α) ($p<0.01$) and uncoupling protein 1 (UCP1) ($p<0.001$) (increase in 2x and 6x, respectively) than CC and LC groups. The LO group had the PGC1α gene expression increased (Fig. 3A) when compared to LL group, while LC showed similar values to groups LO and LL ($p<0.01$). The mRNA levels of UCP1 (Fig. 3C) were higher in the LO group, while LC and LL were lower e similar between themselves ($p<0.01$). However, the hormone-sensitive lipase (HSL) gene expression (Fig. 3B) was only influenced by nutritional status ($p<0.01$), wherein the LC and LO groups were lower than CC and CO and higher than LL ($p<0.01$). In relation to protein content, there was no statistical differences of HSL (Fig. 4A), UCP1 (Fig. 4B), β3-adrenergic receptors (ADRB3) (Fig. 4C) and adipose triglyceride lipase (ATGL) (Fig. 4D).

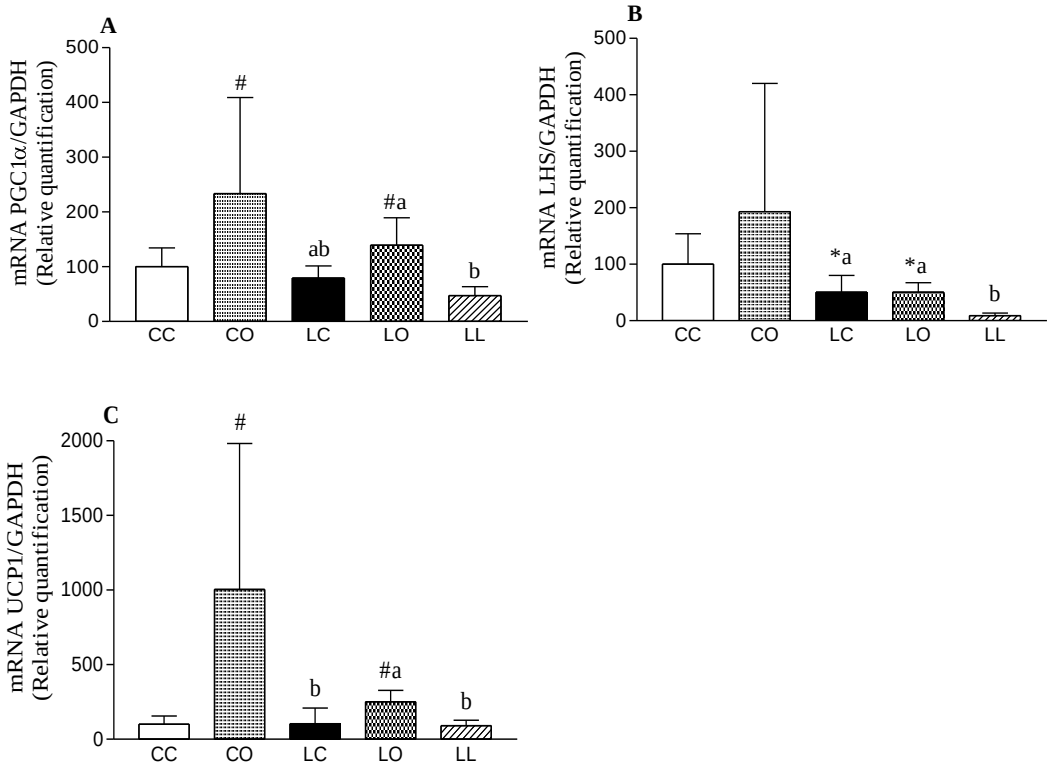

Figure 3. Peroxisome proliferator-activated receptor-γ coactivator - PGC1α (A), hormone-sensitive lipase - HSL (B) and uncoupling protein 1 - UCP1 (C) mRNA analyzed by real-time polymerase chain reaction in IBAT of adult rats maintained on control casein (CC and LC groups), *okara* (CO and LO groups) or low-protein (LL group) diets after weaning. The bars are expressed as the mean ± SD (n=4-5 rats). Means with different superscript uppercase letters are significantly different by two-way analysis of variance, and those with superscript lowercase letters are significantly different by one-way analysis of variance followed by LSD test ($p<0.05$). *indicates the difference between nutritional states, and $^{\#}$indicates the difference between diets.

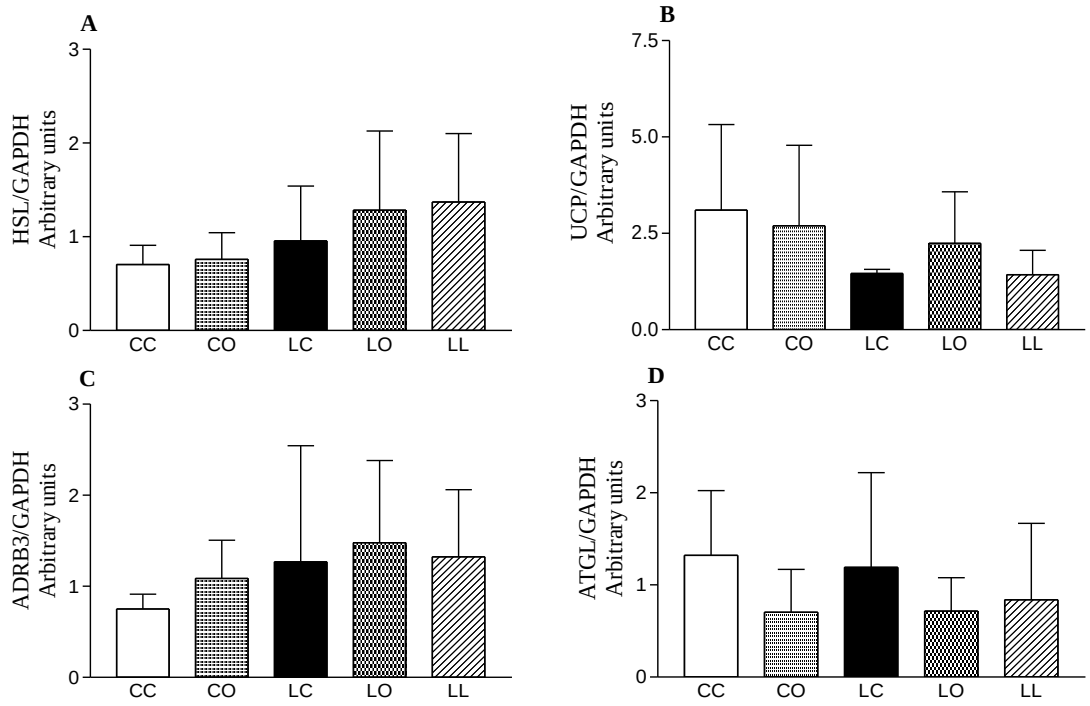

Figure 4. Hormone-sensitive lipase - HSL (A), uncoupling protein 1 - UCP1 (B), β3-adrenergic receptors - ADRB3 (C) and adipose triglyceride lipase - ATGL (D) content IBAT from the adult rats maintained on control casein (CC and LC groups), *okara* (CO and LO groups) or low-protein (LL group) diets after weaning. Equal amounts of protein (80μg) were run on SDS-PAGE. The results were normalized to the content of GAPDH. The bars are expressed as the mean ± SD (n=3-5 rats). Means with different superscript uppercase letters are significantly different by two-way analysis of variance, and those with superscript lowercase letters are significantly different by one-way analysis of variance followed by LSD test ($p<0.05$). *indicates the difference between nutritional states, and $^{\#}$indicates the difference between diets.

A cold tolerance test has confirmed that the thermogenic capacity of the rats was improved by okara diet, independently of nutritional status after 4h test (Figure 5). The body temperature before cold exposure ($F_{1,16}=6.88$, $p<0.05$) and further to 4h ($F_{1,16}=16.34$, $p<0.001$) was higher in the animals fed with *okara* diet compared with animals treated with casein. Furthermore, with 4h exposure to cold, animals LO managed to kept the body temperature higher than the LL and LC groups, which were similar among themselves ($p<0.001$). After 3h of test, independently of diet, groups recovered from the malnutrition maintained higher body temperature in relation to controls ($F_{1,16}=6.40$, $p<0.05$). At the end of cold exposure (5h), the LO group had higher body temperature than CC, CO and LC groups, and these were similar among

themselves ($F_{1,16}$=5.66, p<0.05). Additionally, when compared to the group LL, the LO kept greater body temperature, followed by LC ($F_{2,10}$=36.51, p<0.0001).

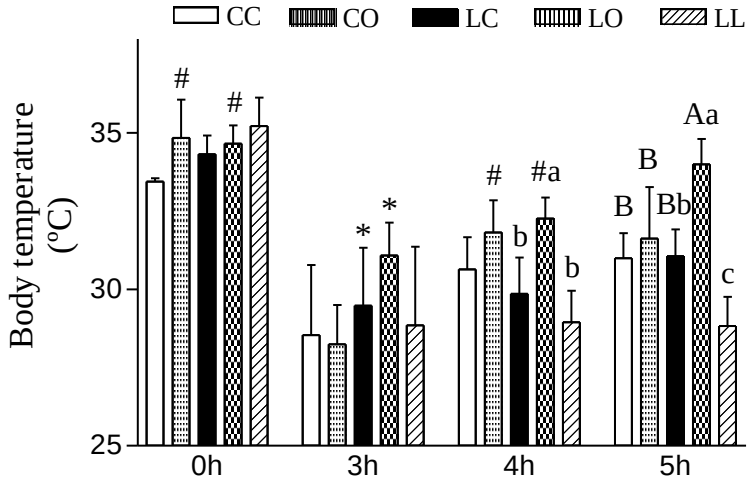

Figure 5. Cold tolerance test on adult rats maintained on a control casein (CC and LC groups), control *okara* (CO and LO groups), or low-protein (LL group) diet after weaning. The bars are mean ± SD (n=3-5 rats). Means with different superscript uppercase letters are significantly different by two-way analysis of variance, and those with superscript lowercase letters are significantly different by one-way analysis of variance followed by LSD test (p<0.05). *indicates the difference between nutritional states, and #indicates the difference between diets.

As the animals treated with okara consume more lipids and the nutritional recovery decreased the EWAT and RWAT weight, we carried out analyzing ex vivo lipolysis of those animals (Figure 6). Rats fed with *okara* diet after weaning, presented significantly higher rates of lipolysis stimulated (+ isoproterenol) than the animals treated with casein under the same condition ($F_{1,14}$=4.93, p<0.05). However, in the RWAT had no statistical differences among the groups, both baseline and stimulated state (Figure 6B). The ratio pLHS/LHS total in stimulated EWAT was similar among all groups (Figure 6C).

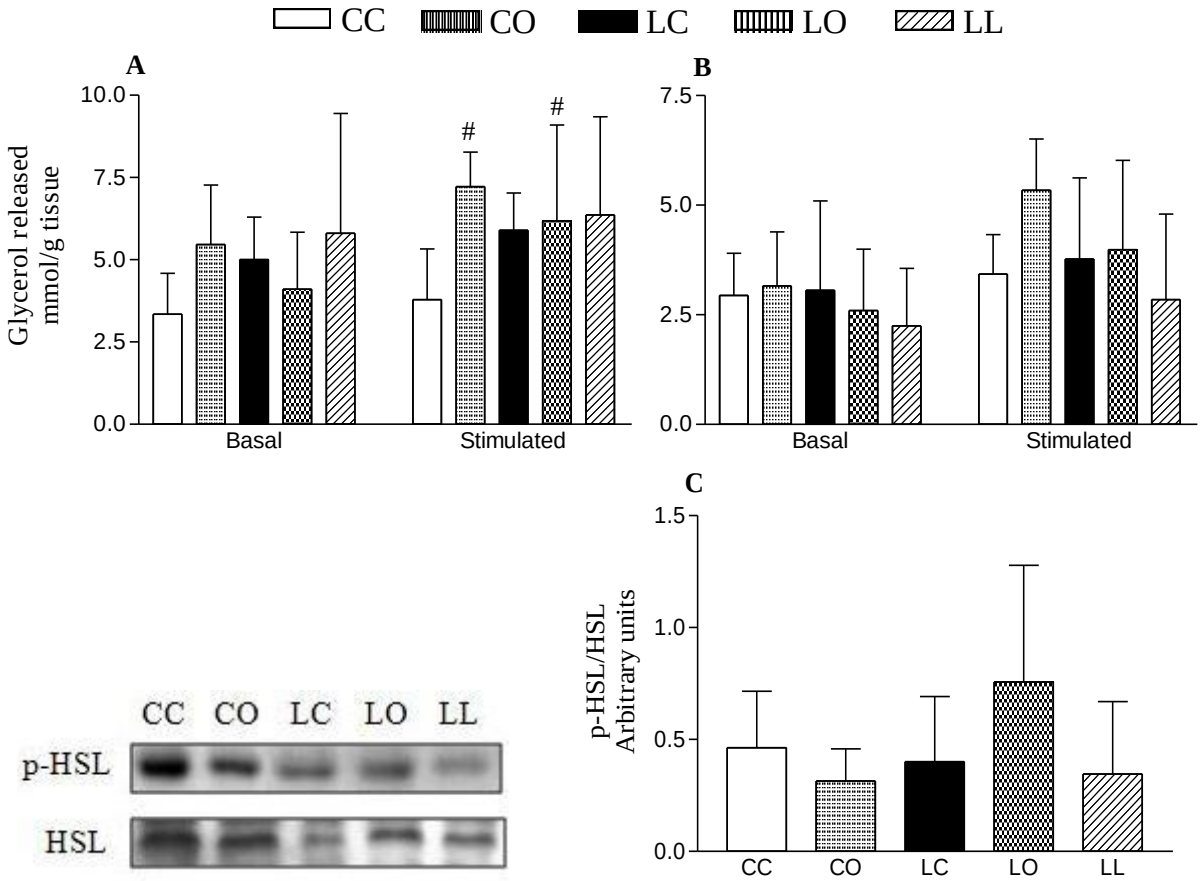

Figure 6. *Ex vivo* lipolysis on EWAT (A), RWAT (B) and phosphorylation of HSL (C) in the stimulated EWAT on adult rats maintained on a control casein (CC and LC groups), *okara* (CO and LO groups), or low-protein (LL group) diet after weaning. The bars are mean ± SD (n=3-5 rats). Means with different superscript uppercase letters are significantly different by two-way analysis of variance, and those with superscript lowercase letters are significantly different by one-way analysis of variance followed by LSD test ($p<0.05$). *indicates the difference between nutritional states, and $^{\#}$indicates the difference between diets.

DISCUSSION

The food intake has many important functions that involve the providing of calories to supply energy needs and amino acids to protein synthesis. When there is protein deficiency in diet, the food intake tends to be higher in order to obtain sufficient amounts of these macronutrients to sustain protein synthesis (Lowel & Spiegelman, 2000) and for animals LL, in spite of consuming less grams of diet, when we corrected to the weight, this consumption is significantly higher than the recovered animals, demonstrating that recovery was able to decrease hyperphagia commonly observed in

malnourished animals. Furthermore, it was observed in our LL animals, lower oxygen consumption and spontaneous activity and according to literature, the low protein intake (around 6% casein) is also related to the decrease of basal metabolism (Horst, Mendel & Benedict, 1933). Another important factor to be considered is that the basal metabolism is not linear even when compared with animals from the same specie, due to be influenced besides other factors, by age, under or over-feeding, total body mass, height and specific metabolic rate of each organ (Even et al., 2001). In relation to group LO, we can infer that its lower spontaneous activity contributed to a reduction of your VO_2, however we cannot say the same for the CO group, which showed activity pattern similar to the CC and still lower VO_2. Although serum levels of THs found in the study did not explain the reduction of VO_2 CO and LO animals, they can also influence the oxygen consumption through the personalized signaling in some organs. In this case, the activation of the metabolic effects THs depends on the deiodination pathways that occur inside the target cells, which were non evaluated in this study and are apparently invisible from the viewpoint of plasma (Bianco, 2011).

Our data shown that fiber intake 2x higher in animals treated with *okara* was effective in increasing the fecal moisture, decreasing the cecal pH and increasing the total intestinal weight, even with relative intestinal length similar to the other groups and this effect can be attributed to the soluble fibers fermentation (Saad, 2006) and by the mechanical activity of insoluble fiber of this subproduct (Bowles & Demiate, 2006). This intestinal fermentation generates microbial metabolites, such as short chain fatty acids (Préstamo et al., 2007), that in addition to altering the intestinal pH, can also influence in the nutrients bioavailability (Venema, 2010). Studies using different diets supplementation with *okara* have found: increased use of dietary fiber, less iron absorption (Matsuo, 1996) and higher apparent absorption and real retention of calcium (Jiménez-Escrig et al., 2008).

Furthermore, in this study, we have shown that *okara* was capable of reducing the total dietary intake, the body weight gain and additionally when administered to animals submitted to protein restriction it was effective in suppressing the EWAT and RWAT weight. When investigating lipolysis in these tissues, we demonstrated that possibly the animals treated with okara are more sensitive to stimulants ADRB3 only in EWAT, although the p-HSL/HSL ratio do not explain the increase of lipolysis stimulated, it is possible that has an alteration in other lipolysis step, such as ATGL of

activation that is an event also important to the mobilization from TAG (Lass et al., 2011). In 3T3-L1 cells isoproterenol-stimulated the overexpression of ATGL increased release of free fatty acids and glycerol, while the ATGL siRNA had the opposite effect (Kershaw et al., 2006). The two analyzed fat stores may respond metabolically different to fat of the diet (Gaíva et al., 2001), this may be attributed the different functions of these tissues in relation energy metabolism. Is proposed that in the EWAT occurs a buildup of essential fatty acids dietary and as a result of the suppression of lipogenesis by steroid hormones we have the supply of polyunsaturated fatty acids for the epididymis. Thus, while the EWAT seems to regulate metabolism site, appears to respond RWAT for the maintenance of energy homeostasis corporal (Caesar et al., 2010).

Animals fed *okara* consumed more lipids due to diet composition and possibly this factor has contributed to reduction of food intake, because it can stimulate the increase the secretion of cholecystokinin which is a hormone related to the process of satiety (Maggio et al., 1988; Woods & D'Alessio, 2008). Additionally, studies show that high fat content feed generates diet-induced thermogenesis (Young et al., 1982) and increased tolerance to cold (Qiu et al., 2001). Animals fed okara diet showed increased cold tolerance, increased body temperature even before the test and despite showing larger expression of mRNA from pro-thermogenic genes in IBAT, we did not find the same result for the protein content of HSL, ATGL, ADRB3 and UCP1 in this tissue.

In conclusion, the present study *in vivo* demonstrated that the *okara* diet possibly has reduced energy expenditure, but was effective in increases tolerance cold, in weight control and fat deposits reduction in animals recovered from malnutrition, besides having beneficial intestinal effects that are independent of the nutritional status.

Acknowledgments

The authors are grateful to Celso Roberto Afonso for his excellent technical assistance. This work was supported by the Brazilian foundations CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico), FAPEMAT (Fundação de Amparo à Pesquisa do Estado de Mato Grosso) and CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior). This work is part of a dissertation presented by Faena Moura de Lima as a partial requirement for the Master’s degree in Biosciences at the College of Nutrition, UFMT.

Contributors

FML, APCA, SRLR, LFRN, and KCS carried out the conduced experiments. FML and RVV contributed equally to this paper in various aspects of this study. RVV and MQL conceived of the study and designed this study. FML drafted the manuscript along with the other authors. All authors read and approved the final manuscript. The authors have no conflict of interest.

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

Ahmed LA, Hassan DR & Hemeda HM. Anti-hyperglycemic effects of okara, corn hull and their combination in alloxan induced diabetic rats. World App Sci J 2010; 9(10): 1139–47.

AOAC Method 923.03. In official methods of analysis. Arlington: AOAC International 1995; 16ªed.

Bianco AC. Minireview: cracking the metabolic code for thyroid hormone signaling. Endocrinology 2011; 152(9): 3306–11.

Bowles S & Demiate IM. Caracterização físico-química de *okara* e aplicação em pães do tipo francês. Ciênc Tecnol Aliment 2006; 26(3): 652-59.

Caesar R, Manieri M, Kelder T, Boekschoten M, Evelo C, et al. A combined transcriptomics and lipidomics analysis of subcutaneous, epididymal and mesenteric adipose tissue reveals marked functional differences. PLoS ONE 2010; 5(7): 1-14.

Chen PH, Chen GC, Yang MF, Hsieh CH, Chuang SH, Yang HL, et al. Bitter melon seed oil–attenuated body fat accumulation in diet-induced obese mice is associated with camp-dependent protein kinase activation and cell death in white adipose tissue. J Nutr 2012; 142(7): 1197-204.

Even PC, Rolland V, Roseau S, Bouthegourd JC, Tomé D. Prediction of basal metabolism from organ size in the rat: relationship to strain, feeding, age and obesity. Am J Physiol Regul Integr Comp Physiol 2001; 280(6): 1887-96.

Gaíva MH, Couto RC, Oyama LM, Couto GE, Silveira VL, Riberio EB, Nascimento CM. Polyunsaturated fatty acid rich diets: effect on adipose tissue metabolism in rats. Br J Nutr 2001; 86 (3): 371–7.

Hales CN & Barker DJP. The thrifty phenotype hypothesis. Br Med Bull 2001; 60(1): 5-20.

Horst K, Mendel LB, Benedict FG. The influence of previous diet, growth and age upon the basal metabolism of the rat. J Nutr 1934; 8(2): 139-62.

Jiménez-Escrig A, Tenorio MD, Espinosa-Martos I & Rupérez P. Health-promoting effects of a dietary fiber concentrate from the soybean byproduct okara in rats. J Agric Food Chem 2008; 56(16): 7495–501.

Katayama M & Wilson LA. Utilization of okara, a byproduct from soymilk production, through the development of soy-based snack food. J Food Sci 2008; 73(3): 152-7.

Kershaw EE, Hamm JK, Verhagen LAW, Peroni O, Katic M, Flier JS. Adipose triglyceride lipase: function, regulation by insulin, and comparison with adiponutrin. Diabetes 2006; 55 (1):148-57.

Lass A, Zimmermann R, Oberer M, Zechner R. Lipolysis – A highly regulated multi-enzyme complex mediates the catabolism of cellular fat stores. Prog Lipid Res 2011; 50(1): 14–27.

Latorraca MQ, Reis MAB, Carneiro EM; Mello MAR; Velloso LA; Saad MJA, *et al*. Protein deficiency and nutritional recovery modulate insulin secretion and the early steps of insulin action in rats. J Nutr 1998; 128(10): 1643-49.

Lescano CAA, Rocha SCS. Drying of the soymilk residue okara in spouted bed. In: ENPROMER- 2nd Mercosur Congress on Chemical Engineering and 4th Mercosur Congress on Process Systems Engineering; 2005; Rios das Pedras – RJ. p.1–10.

Lowell BB & Spiegelman BM. Towards a molecular understanding of adaptive thermogenesis. Nature 2000; 404(6778): 652-60.

Maggio CA, Haraczkiwicz E & Vasselli JR. Diet composition alters the satiety effect of cholecystokinin in lean and zucker rats. Physiol Behav 1988; 43(4): 485-91.

Matsumoto K, Watanabe Y & Yokoyama S. Okara, soybean residue, prevents obesity in a diet induced murine obesity model. Biosc Biotech Bioch 2007; 71(3): 720-27.

Matsuo M. Acceleration of sterol excretion, little meteorism and less inhibition of iron absorption by *okara koji*, a new foodstuff, in rats. Biosc Biotech Bioch 1996; 60 (4): 571-74.

Mattson MP. Perspective: does brown fat protect against diseases of aging? Ageing Res Rev 2010; 9(1): 69-76.

Nakornpanom NN, Hongsprabhas P, Hongsprabhas P. Effect of soy residue (okara) on in vitro protein digestibility and oil release in high-calorie emulsion stabilized by heated mixed proteins. Food Research International 2010; 43(1): 26–32.

O'Toole DK. Characteristics and use of okara, the soybean residue from soy milk productions: a review. J Agric Food Chem 1999; 47(2): 363-71.

Patel MS & Srinivasan M. Metabolic programming: causes and consequences. J Biol Chem 2002; 277(3): 1629-32.

Préstamo G, Rupérez P, Espinosa-Martos I, Villanueva MJ, Lasunción MA. The effects of okara on rat growth, cecal fermentation, and serum lipids. Eur Food Res Technol 2007; 225(5-6): 925–28.

Proscky L, Aps NG, Furda I, Devries JW, Schweizer TF, Harland BF. Determination of total dietary fiber in foods, products and total diets: Interlaboratorial Study. J Assoc Off Anal Chem 1984; 67(6): 1044–52.

Qiu J, Ogus S, Lu R & Chehab FF. Transgenic mice overexpressing leptin accumulate adipose mass at an older, but not younger, age. Endocrinology 2001; 142(1): 348-58.

Reeves PG, Nielsen FH, Fahey CG. AIN-93 purified diets for laboratory rodents: reports of the American Institute of Nutrition ad hoc working committee on the reformulation of the AIN-76 rodent diet. J Nutr 1993; 123(11): 1939-51.

Saad SMI. Probióticos e prebióticos: o estado da arte. Rev Bras Cienc Farm 2006; 42(1): 1-16.

Sartorelli DS & Franco LJ. Tendências do diabetes mellitus no Brasil: o papel da transição nutricional. Cad Saúde Pública 2003: 19 Suppl 1: 29-36.

Sokal RR, Rohlf FJ. Assumptions of analysis of variance. In: Sokal RR, Rohlf FJ, editors. Biometry: the principles and practice of statistics in biological research. New York: WH Freeman & Co. 1995; 392–450.

Villanueva AMJ, Yokoyama BWH, Hong BYJ, Barttley BGE, Rupérez CP. Effect of high-fat diets supplemented with okara soybean by-product on lipid profiles of plasma, liver and faeces in Syrian hamsters. Food Chemistry 2011; 124(1): 72–79.

Woods SC & D'Alessio DA. Central Control of Body Weight and Appetite. J Clin Endocrinol Metab 2008; 93(11): S37–S50.

Yokomizo A, Takenaka Y & Takenaka T. Antioxidative activity of peptides prepared from okara protein. Food Sci Technol Res 2002; 8(4): 357–59.

Young JB, Saville E, Rothwell NJ, Stock MJ, Landsberg L. Effect of diet and cold exposure on norepinephrine turnover in brown adipose tissue of the rat. J Clin Invest 1982; 69(5): 1061–71.

Zenebon O, Pascuet NS (Coord.). Normas analíticas do Instituto Adolfo Lutz. 4.ed. São Paulo: Instituto Adolpho Lutz 2005; 1: 1020p.