UNIVERSIDADE FEDERAL DE MATO GROSSO
FACULDADE DE NUTRIÇÃO
PROGRAMA DE PÓS-GRADUAÇÃO EM NUTRIÇÃO, ALIMENTOS E METABOLISMO

Estado ácido-base, função renal e perfil metabólico em ratos alimentados durante a fase de crescimento após o desmame com uma dieta a base de caseína e óleo de coco

Caroline Corrêa Menezes

Cuiabá-MT,
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1. Doutora Márcia Queiroz Latorraca (Presidente Banca)
INSTITUIÇÃO:UNIVERSIDADE FEDERAL DE MATO GROSSO
2. Doutora Márcia Queiroz Latorraca (Orientadora)
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4. Doutora Marise Auxiliadora de Barros Reis (Examinadora Interna)
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LISTA DE ABREVIATURAS

ABESO	Associação brasileira para estudo de obesidade e síndrome metabólica
AcAc	Aceto acetato
AGCL	Ácidos graxos de cadeia longa
AGCM	Ácidos graxos de cadeia média
AGLs	Ácidos graxos livres
ALT	Alanina aminotransferase ou alanine transaminase
AST	Aspartatoaminotransferase ou aspartate transaminase
ATP	Trifosfato de adenosina
AUGC	Área sob a curva da glicose ou área under the glucose curve
BHB	Beta-hidroxibutirato
BW	Body Weight ou peso corporal
CO	Coconut oil
EWAT	Epididymal white adipose tissue ou tecido adiposo branco epididimal
FPR	Fluxo plasmático renal
HLD	High Density Lipoprotein ou lipoproteína de alta densidade
IBAT	Brown adipose tissue ou tecido adiposo marrom
ITT	Teste de tolerância à insulina
K_{ITT}	The glucose decrease constant ou constante de decaimento da glicose
LCFA	Long chain fatty acids
LDL	Low Density Lipoproteins ou Lipoproteínas de baixa densidade
MCFA	Medium chain fatty acids
OC	Óleo de coco
OGTT	Teste oral de tolerância à glicose ou oral glucose-tolerancetest

OS	Óleo de soja
PWAT	Perirenal white adipose tissue ou tecido adiposo branco perirenal
RWAT	Retroperitoneal white adipose tissue ou tecido adiposo branco retroperitoneal
SBCAL	Brazilian Society for Science in Laboratory Animals
SBEM	Sociedade brasileira de endocrinologia e metabolismo
TFG	Taxa de filtração glomerular
VLDL	Very Low Density Lipoproteins ou Lipoproteínas de muito baixa densidade

RESUMO

Introdução: O óleo de coco emergiu como uma gordura capaz de promover benefícios à saúde; no entanto, pelo alto conteúdo de triglicerídeos da cadeia média, é um potencial produtor de corpos cetônicos e, consequentemente, acidose metabólica média. As dietas ocidentais nos países industrializados contêm principalmente produtos de origem animal, indutores de acidose e consequentemente produzem um estado de acidose metabólica. A má adaptação do corpo às dietas acidogênicas parece contribuir para a patogênese de doenças crônicas como doenças cardiovasculares, metabólicas e renal. **Objetivo:** Avaliamos os efeitos do uso de óleo de coco associado a uma dieta potencialmente acidificante contendo proteína de origem animal sobre o estado ácido-base, função renal e metabolismo de ratos alimentados durante a fase de crescimento após o desmame. **Métodos:** Foram avaliados três grupos diferentes: SO, constituído por ratos alimentados com dieta controle (AIN-93G); SO + OC, composto por ratos alimentados com uma dieta contendo uma mistura de óleo de soja e óleo de coco; e OC, composto pelos ratos alimentados com uma dieta contendo óleo de coco. **Resultados:** A ingestão total e relativa de alimentos, bem como a ingestão cumulativa de água foram maiores nos grupos CO em comparação aos grupos SO e SO + CO. O peso corporal não diferiu entre os grupos, mas o peso do tecido adiposo branco epididimal em g e g/100g de peso corporal foi maior no grupo CO do que no grupo SO e não diferiu no grupo SO + CO. Os corpos cetônicos e glicemia sérica pós-prandial e em jejum não diferiram entre os grupos. As concentrações plasmáticas de lactato no estado pós-prandial e em jejum foram menores no grupo CO do que nos grupos SO, e no grupo SO + CO foram semelhantes aos grupos SO e SO + CO. A concentração de glicerol sérico pós-prandial foi maior no grupo CO em comparação ao grupo SO e semelhante ao grupo SO + CO. A concentração sérica de glicerol no estado de jejum não diferiu entre os grupos. Os

parâmetros gasométricos não diferiram nos três grupos, mas no grupo CO o hiato aniônico foi menor no grupo SO, e no grupo SO + CO o hiato aniônico não diferiu nos grupos SO e CO. O clearance osmolar e de água e a uréia urinária foram maiores no grupo CO do que nos grupos SO, mas foram semelhantes ao grupo SO + CO. O clearance de creatinina, área glomerular e área do corpúsculo glomerular foram maiores nos grupos CO e SO + CO do que no grupo SO. A excreção fracionada de sódio foi menor no grupo SO + CO em comparação ao grupo SO e não diferiu entre os grupos CO e SO. A concentração total de proteína e globulina no soro foi maior no grupo CO em comparação ao grupo SO; O grupo SO + CO não diferiu dos grupos CO ou SO. A concentração sérica de albumina e Kitt foram menores no grupo SO + CO do que nos grupos SO e CO, enquanto os níveis séricos de ALT no SO + CO e CO foram semelhantes e ambos foram menores em comparação ao grupo SO. O nível sérico de AST foi menor no grupo SO + CO do que no grupo SO, e o grupo CO não diferiu de ambos. Triglicérides séricos, concentrações de VLDL e HDL foram maiores no grupo CO em comparação aos grupos SO + CO e SO. A concentração sérica de colesterol total foi semelhante nos grupos SO + CO e CO e ambos foram maiores em comparação ao grupo SO. O nível sérico de LDL e o índice aterogênico foram maiores no grupo SO e CO do que nos grupos CO e SO. **Conclusão:** Assim, a administração de uma dieta à base de caseína contendo óleo de coco durante o crescimento do desmame não alterou o estado ácido-base, mas prejudicou a estrutura e a função renal e contribuiu para um perfil aterogênico na idade adulta.

Palavras-chave: óleo de coco, dieta cetogênica, estado ácido-base, função renal, metabolismo, ratos

ABSTRACT

Coconut oil has emerged as a fat capable of promoting health benefits, however, by the high medium chain triglycerides content, is a potential producer of ketone bodies and consequently of middle metabolic acidosis. Western diets in industrialized countries contains mainly animal products, that is acid inducing and consequently produces a state of metabolic acidosis. Poor adaptation of the body to acidogenic diets seems to contribute to the pathogenesis of chronic diseases such as cardiovascular, metabolic and renal. We evaluated effects of coconut oil use associated with a potentially acidifying diet containing protein of animal origin on acid-base status, renal function and metabolism rats fed during the growing phase after weaning. It was evaluated three different groups: SO, consisting of rats fed a control diet (AIN-93G); SO+OC, consisting of rats fed a diet containing a mix soybean oil and coconut oil; and OC, consisting of the rats fed a diet containing coconut oil. The total and relative food intakes as well as cumulative water intake were higher in the CO groups compared to SO and SO+CO groups. Body weight did not differ among groups, but the epididymal white adipose tissue weight in g and g/100g of body weight was higher in the CO group than in the SO group, and did not differ to SO+CO group. The post-prandial and fasting blood glucose and ketone bodies did not differ among groups. Plasma lactate concentrations at post-prandial and fasting status were lower in the CO group than in the SO groups, and in the SO+CO group were similar to SO and SO+CO groups. The post-prandial serum glycerol concentration was higher in the CO group compared to SO group, and similar to SO+CO group. Serum glycerol concentration at fasting status did not differ among groups. Gasometry parameters did not differ in the three groups, but in the CO group anion gap was lower in than in SO group, and in the SO+CO group anion gap did not differ to SO and CO groups. Osmolar and water clearances, urinary urea

were higher in the CO group than in the SO groups, but were similar to SO+CO group. The creatinine clearance, glomerular area, glomerular corpuscle area were higher in the CO and SO+CO groups than in the SO group. Fractional excretion of sodium was lower in the SO+CO group compared to SO group and did not differ between CO and SO groups. Serum total protein and globulin concentration were higher in the CO group compared to SO group; SO+CO group did not differ to CO or SO groups. Serum albumin concentration and K_{itt} were lower in SO+CO group than in the SO and CO groups, whereas serum ALT levels in the SO+CO and CO were similar and both were lower compared to SO group. Serum AST level was lower in the SO+CO group than in SO group, and CO group did not differ to both. Serum triglicerydes, VLDL and HDL concentrations were higher in the CO group compared to SO+CO and SO groups. Serum total cholesterol concentration was similar in SO+CO and CO groups and both were higher compared to SO group. Serum LDL level and atherogenic index were higher in the SO and CO group than in CO and SO groups. Thus, the administration of a casein-based diet containing coconut oil during weaning growth did not alter the acid-base state, but impaired renal structure and function and contributed to an atherogenic profile in adulthood.

Keywords: coconut oil, cetogenic diet, acid-base status, renal function, metabolism, rats

1. INTRODUÇÃO

A dieta é um dos principais determinantes da carga ácida excretada pelo rim para manter o equilíbrio ácido-base (Gonick et al. 1968). Durante a evolução humana as dietas continham predominantemente frutas e vegetais que são alcalinizantes. Nas sociedades contemporâneas industrializadas as dietas são deficientes em frutas e vegetais e contêm excesso de produtos de origem animal que são potencialmente acidificantes (Sebastian et al. 2002; Strohle et a. 2010; Remer et al. 2003).

As frutas e os vegetais possuem altas concentrações de potássio e ânions metabolizáveis (citrato e malato) que alcalinizam o meio, porque quando metabolizados utilizam íons hidrogênio. As proteínas vegetais são ricas em glutamato (aminoácidos aniônico), que ao ser metabolizado também consome íon hidrogênio para se tornar neutro. Por outro lado, os aminoácidos sulfurados (metionina, homocisteína e cistina) encontrados nos cereais e proteínas animais, ao serem oxidados produzem sulfato (ânion não metabolizável), considerado o principal determinante da carga ácida da dieta. Além disso, nos alimentos de origem animal e nos cereais, os ânions que acompanham o potássio são principalmente fosfato e cloreto, o que tornam esses alimentos mais acidogênicos do que as frutas e vegetais (Hunt 1956; Bresslau et al. 1988; Maurer et al. 2003; Demigne et al. 2004; Jehle et al. 2006). A má adaptação do organismo as dietas acidogênicas parece contribuir para a patogênese de doenças crônicas como as cardiovasculares, metabólicas e renais (Cordain et al. 2005).

Em resposta à acidose metabólica induzida por dieta, os rins removem ânions não metabolizáveis, conserva citrato, aumenta a amoniogênese e a excreção urinária de íons amônio para restaurar o equilíbrio ácido-base. O resultado da ativação desses mecanismos compensatórios é a redução do pH e alteração da composição urinária (baixa concentração de citrato, alta concentração de cálcio, nitrogênio e fosfato). O pH

urinário ácido predispõe à formação de cálculos de ácido úrico, enquanto a hipocitratúria e hipercalciúria são fatores de risco para cálculos de cálcio (Adeva & Souto, 2011). Para remover o excesso de carga ácida, ocorre aumento do fluxo plasmático renal (FPR), da taxa de filtração glomerular (TFG) (Györke et al. 1991; Sartorius et al. 1949; Sulyok & Guignard 1990). A acidose metabólica produz hipertrofia renal, presumivelmente devido ao aumento da amôniogênese renal (Garibotto et al. 2009).

A acidose metabólica estimula o catabolismo muscular para disponibilizar aminoácidos para a amoniogênese, (Tizianello et al. 1980) e inibe a produção de albumina por ativar a via proteolítica-ubiquitina dependente de ATP (Ballmer et al. 1995; Mitch et al. 1994). Mesmo em grau leve, a acidose metabólica induz resistência muscular à ação da insulina, intolerância à glicose e proteólise que também fornece aminoácidos para a geração de amônio (De Fronzo & Beckles 1979). O aumento da secreção de glicocorticoides e da concentração de cortisol no plasma e na urina em resposta à acidose metabólica exacerba a resistência à insulina, a proteólise e a excreção urinária de amônia (Maurer et al. 2003; Ballmer & Imoberdorf 1995; Waybill et al. 1994). Ambas, resistência à insulina e a acidose metabólica estão relacionadas à hipertensão arterial sistêmica. Resultados de estudos observacionais confirmam uma associação entre marcadores de resistência à insulina e acidose metabólica (bicarbonato sérico e pH urinário baixos, *anion gap* sérico alto, hipocitratúria) (Farwell & Taylor 2008). Finalmente, a acidose metabólica crônica está associada à perda óssea por induzir a calciúria devido a uma combinação de efeitos físico-químicos no mineral ósseo e na ativação da reabsorção óssea osteoclástica (Krieger et al. 1992).

Dietas ricas em gordura e pobres em carboidratos também são acidogênicas e estão associadas à alteração no estado ácido-base do organismo por estimularem a lipólise e aumentarem cetonemia (Yancy et al. 2007).

Os corpos cetônicos acetoacetato (AcAc), acetona e β-hidroxibutirato (βHB) são produzidos no fígado em situações fisiológicos, mais comumente durante o jejum prolongado e inanição. A concentração de corpos cetônicos no sangue reflete o equilíbrio entre a produção hepática (cetogênese) e a degradação e utilização (cetólise) periférica, em tecidos extra-hepáticos (Robinson & Williamson, 1980; Laffel, 1999). O substrato primário para a cetogênese são os ácidos graxos livres (AGLs) liberados do tecido adiposo e os aminoácidos cetogênicos (leucina, lisina, fenilalanina, isoleucina, triptofano e tirosina) que contribuem com menos de 5% dos corpos cetônicos circulantes (Thomas et al. 1982). A razão glucagon/insulina elevada e o declínio da concentração hepática de glicogênio estimulam, enquanto a redução do fluxo sanguíneo hepático ou o aumento de corpos cetônicos suprimem a cetogênese (Robinson & Williamson, 1980; Laffel, 1999). A cetogênese é uma resposta adaptativa conservada evolutivamente, que garante a sobrevivência em períodos de jejum prolongado e inanição, porque fornece ao cérebro substrato energético, uma vez que este não utiliza ácidos graxos como fonte de energia.

A cetólise ocorre principalmente no coração e rins, seguido pelo músculo esquelético e o cérebro (Robinson e Williamson, 1980). Como o músculo esquelético corresponde a 40% da massa corporal em humanos adultos, esse órgão utiliza a maior fração de corpos cetônicos no repouso (Balasse & Fery, 1989; Laffel, 1999). O músculo esquelético tem uma alta afinidade pelos corpos cetônicos, mas devido às concentrações circulantes baixas a contribuição dos corpos cetônicos para o fornecimento de energia no músculo é inferior a 5%, e os AGLs são a principal fonte de fornecimento de energia

em condições normais. No período pós-prandial, a cetonemia é <0,1 mmol/L e a hipercetonemia é caracterizada por valores superiores a 0,2 mmol/L (Robinson & Williamson,1980).

Os corpos cetônicos são fontes alternativas de energia, atenuam a utilização de glicose nos tecidos periféricos e a proteólise no músculo esquelético, e reduzem a lipólise no tecido adiposo (Robinson & Williamson, 1980). São também considerados terapêuticas alternativas para várias doenças, como as neurodegenativas, as miopatias genéticas, os estados hipóxicos, e nas alterações do metabolismo da glicose (Veech, 2004). A ingestão de ésteres de cetona ou sais de βHB, usada como estratégia nutricional para produzir cetose aguda altera a resposta metabólica e melhora o desempenho durante o exercício (Cox et al. 2016).

A cetonemia também é aumentada após a ingestão de triglicerídeos de cadeia média que fornece ácidos graxos saturados contendo de seis a doze carbonos. Os ácidos graxos de cadeia média (AGCM) são cetogênicos porque são metabolizados mais rapidamente que os de cadeia longa (> 14 carbonos). Diferente dos ácidos graxos de cadeia longa (AGCL), que são absorvidos pelo sistema linfático e incorporados nos quilomícrons na circulação antes de atingir o fígado, os ácidos graxos contendo de oito a dez carbonos atingem o fígado diretamente pela veia porta (Cunnane et al. 2016; Bach & Babayan 1982; Seaton et al. 1986; Papamandjaris et al. 1998; Marten et al. 2006; Schonfeld & Wojtczak 2016). Os AGCM contendo oito carbonos também atravessam a membrana interna mitocondrial sem transporte dependente de carnitina, sendo, portanto, mais rapidamente b-oxidados em comparação com os AGCL (Marten et al. 2006).

Vários AGCM estão presentes no leite de mamíferos, no óleo de palma e óleo de coco (OC) (Henderson 2008; Page et al. 2009; Courchesne-Loyer et al. 2013; Courchesne-Loyer et al. 2015).

O OC é o principal produto obtido do fruto de uma palmeira (*Cocos nucifera*), típica de áreas tropicais e subtropicais. Existem dois tipos de OC: o copra refinado, branqueado e desodorizado; e o óleo virgem. Ambos possuem perfil de ácidos graxos e triglicerídeos semelhante, mas OC virgem possui teor mais alto de compostos bioativos, como vitamina E, esteróis e polifenóis, uma vez que o refino remove uma porção desses compostos (Marina et al., 2009). O OC é composto majoritariamente por ácidos graxos saturados (92%), dos quais 62% correspondem a ácidos graxos com oito a doze carbonos (Eyres et al., 2016). A proporção de ácidos graxos saturados, monoinsaturados e poliinsaturados no OC e no óleo de soja (OS), um dos principais óleos consumidos na dieta está descrita na Tabela 1. Observa-se que o ácido láurico predomina na composição do OC. Há controvérsias quanto à classificação do ácido láurico como AGCM. Alguns consideram AGCM aqueles com oito a doze carbonos, enquanto outros os definem como tendo de seis a dez átomos de carbono (Sankararaman & Sferra, 2018). Scrimgeour (2005) considera ácidos graxos de cadeia curta ou AGCM aqueles que possuem cadeia com menos de dezesseis átomos de carbono.

Tabela 1. Perfil de ácidos graxos do óleo de coco (OC) e óleo de soja (OS)

Ácido Graxo (%)	OC	OS
Butírico C4:0	-	-
Capróico C6:0	0,5	-
Caprílico C8:0	7,8	-
Cáprico C10:0	6,7	-
Laurico C12:0	47,5	-
Mirístico C14:0	18,1	-
Palmítico C16:0	8,8	10,0
Esteárico C18:0	2,6	4,0
Palmitoleico C 16:1	-	-
Oleico C18:1	6,2	23
Linoleico C18:2	1,6	51
a-Linolênico C18:3	-	7
C20:0	0,1	-
C22:0		
C20:1	Traços	-
Outros	-	5

Fonte: Adaptado de Silva Lima & Block 2019.

Considerando a predominância do ácido láurico na sua composição, o OC, juntamente com os óleos de palma e de babaçu são chamados de óleos láuricos. Por se comportarem de maneira muito diferente no metabolismo em comparação com as gorduras compostas majoritariamente por AGCL, têm sido atribuídas propriedades únicas aos óleos láuricos, que incluem: redução do peso corporal e do colesterol

sanguíneo, prevenção de doenças cardiovasculares, efeitos antimicrobiano, anti-inflamatório e antiviral, produção de corpos cetônicos que são benéficos na doença de Alzheimer (Dayrit, 2014, Dayrit 2015).

Entretanto, por possuir em sua composição predominantemente ácidos graxos saturados, cuja ingestão excessiva correlaciona-se com o aumento das lipoproteínas de baixa densidade e do risco cardiovascular, ainda não há consenso quanto aos benefícios do OC. O Departamento de Agricultura dos Estados Unidos (USDA, 2015), a Organização Mundial da Saúde (WHO, 2018) e a Autoridade Europeia para Segurança dos Alimentos (EFSA, 2017) recomendam que a ingestão diária de gorduras saturadas não ultrapasse 10% do total de calorias ou que o consumo dessas gorduras seja o mais baixo possível. No Brasil, o quarto maior produtor de OC, a Sociedade Brasileira de Endocrinologia e Metabolismo (SBEM) e a Associação para o Estudo da Obesidade e Síndrome Metabólica (ABESO) não endossam o uso desse produto visando perda de peso, e recomendam o seu uso de forma restrita (SBEM, ABESO 2015).

Diante do exposto, ficam claras as controvérsias entre veículos da mídia e especialistas em saúde que garantem que o OC é capaz de promover benefícios à saúde, e as agências reguladoras governamentais de muitos países, céticas quanto aos seus benefícios, devido ao teor elevado de ácidos graxos saturados. Portanto, estudos que avaliem os efeitos da utilização dessa gordura associada a dietas potencialmente acidificantes contendo proteína de origem animal podem contribuir para esse debate.

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3. OBJETIVOS

3.1 Objetivo geral:

Avaliar o estado ácido-base, a função renal e o metabolismo em ratos adultos alimentados durante a fase de crescimento após o desmame com uma dieta à base de caseína e OC.

3.2 Objetivos específicos:

Determinar em ratos adultos alimentados durante a fase de crescimento após o desmame com uma dieta à base de caseína e OC:

- o padrão de ingestão alimentar e hídrica;
- o perfil nutricional;
- o estado de hidratação;
- a concentração de corpos cetônicos no sangue;
- o estado ácido-base;
- a função renal;
- a função hepática;
- o perfil lipídico;
- a sensibilidade à insulina;
- a tolerância à glicose.

4. ARTIGO

A casein-based diet containing coconut oil during weaning growth impaired renal structure and function and contributed to an atherogenic profile in adulthood

Caroline Corrêa Menezes[1], Leonardo Marin[2], Carolina Sólon da Silva[4], Amílcar Sabino Damazo[3], Chaiane Aline da Rosa[4], Luana Resende Silva[4], Márcia Queiroz Latorraca[4]

[1]Mestrado em Nutrição, Alimentos e Metabolismo, Faculdade de Nutrição, Universidade Federal de Mato Grosso, Cuiabá-MT, Brasil.

[2]Governo do Estado de Mato Grosso, Hemocentro, Cuiabá-MT, Brasil.

[3]Departamento de Ciências Básicas da Saúde, Faculdade de Medicina, Universidade Federal de Mato Grosso, Cuiabá-MT, Brasil.

[4]Departamento de Alimentos e Nutrição, Faculdade de Nutrição, Universidade Federal de Mato Grosso, Cuiabá-MT, Brasil.

Corresponding author: Márcia Queiroz Latorraca, Faculdade de Nutrição, Universidade Federal de Mato Grosso (UFMT), Avenida Fernando Correa da Costa, 2367. Bairro Boa Esperança. Cuiabá, MT, Brazil- 78060-900. Phone: +55-65-33137396. Fax: +55-65-36158811. E-mail: mqlator@terra.com.br

Short title: coconut oil impairs renal function

Abstract

Coconut oil has emerged as a fat capable of promoting health benefits; however, due to its high medium-chain triglyceride content, coconut oil is a potential producer of ketone bodies and consequently of middle metabolic acidosis. Western diets in industrialised countries contain mainly animal products, which are acid inducing and consequently produce a state of metabolic acidosis. Poor adaptation of the body to acidogenic diets seems to contribute to the pathogenesis of chronic diseases such as cardiovascular, metabolic and renal diseases. We evaluated the effects of coconut oil use associated with a potentially acidifying diet containing protein of animal origin on the acid-base status, renal function and metabolism in rats fed during the growing phase after weaning. Three different groups were evaluated: SO, consisting of rats fed a control diet (AIN-93G); SO+CO, consisting of rats fed a diet containing a mix of soybean oil and coconut oil; and CO, consisting of rats fed a diet containing coconut oil. The total food intake and cumulative water intake were higher in the CO group than in the SO and SO+CO groups. Body weight did not differ among the groups, but the food intake relative to body weight and the epididymal white adipose tissue weight in g and g/100 g of body weight were higher in the CO group than those in the SO group and did not differ from those in the SO+CO group. The post-prandial and fasting blood glucose and ketone bodies did not differ among groups. Plasma lactate concentrations in the post-prandial and fasting states were lower in the CO group than those in the SO group, and in the SO+CO group, they were similar to those in the SO and CO groups. The post-prandial serum glycerol concentration was higher in the CO group than in both the SO group and the SO+CO group. The serum glycerol concentration in the fasting state did not differ among groups. Gasometry parameters did not differ among the three groups, but in the CO group, the anion gap was lower than that in the SO group, and in the

SO+CO group, the anion gap did not differ between the SO and CO groups. Osmolar and water clearances and urinary urea were higher in the CO group than in the SO group but were similar to those in the SO+CO group. The creatinine clearance, glomerular area, and glomerular corpuscle area were higher in the CO and SO+CO groups than those in the SO group. Fractional excretion of sodium was lower in the SO+CO group than in the SO group and did not differ between the CO and SO groups. Serum total protein and globulin concentrations were higher in the CO group than in the SO group; the SO+CO group did not differ from the CO or SO groups. Serum albumin concentration and K_{itt} were lower in the SO+CO group than in the SO and CO groups, whereas serum ALT levels in the SO+CO and CO groups were similar and both were lower compared to the SO group. The serum AST level was lower in the SO+CO group than in the SO group, and the CO group did not differ from either group. Serum triglyceride, VLDL and HDL concentrations were higher in the CO group than in the SO+CO and SO groups. The serum total cholesterol concentration was similar in the SO+CO and CO groups, and both were higher compared to the SO group. The serum LDL level and atherogenic index were higher in the SO and CO groups than in the CO and SO groups. Thus, the administration of a casein-based diet containing coconut oil during weaning growth did not alter the acid-base state but impaired renal structure and function and contributed to an atherogenic profile in adulthood.

Keywords: coconut oil, ketogenic diet, acid-base status, renal function, metabolism, rats

Introduction

Diet is one of the main factors that may influence the occurrence of low-grade metabolic acidosis. The modern Western diet contains large quantities of animal protein and excess sodium chloride and possesses low fruit and vegetable contents, generating an excessively acidic environment (Adeva and Souto 2011). In response to metabolic acidosis, the kidney implements compensatory mechanisms to restore the acid-base balance, which results in significant functional and structural changes, including an increase in renal plasma flow and the glomerular filtration rate to remove the excessive acid load, as well as kidney hypertrophy due to an increase in renal ammoniagenesis (Gyorke et al. 1991; Sulyok and Guignard 1990; Garibotto et al. 2009), a reduction in urine pH and a change in urine composition, such as hypocitraturia and hypercalciuria, nitrogen and phosphate wasting (Simpson 1983; Bresslau et al. 1988). The clinical consequences of diet-induced metabolic acidosis are a predisposition to kidney stone disease, wasting nitrogen, insulin resistance (De Fronzoand Beckles 1979), and systemic hypertension (Taylor et al. 2007).

Coconut oil (CO) has entered the modern diet due its presumable health benefits, including weight reduction, cholesterol lowering, prevention of cardiovascular diseases, and anti-inflammatory effects, among others. These benefits have been attributed to the predominance of lauric acid, a medium-chain fatty acid (MCFA) that behaves very differently in metabolism (Dayrit 2014). However, MCFAs are ketogenic due to their faster metabolism than long-chain fatty acids (LCFAs). It has been shown that a bolus dose of medium-chain triglycerides likewise can increase ketogenesis even in the presence of high glycogen availability because MCFAs can stream into hepatic mitochondria efficiently, where they are rapidly converted either to ketone bodies or to CO_2 (Hoppel 1982). Moreover, because of the predominant saturated fatty acid content,

whose excessive intake correlates with the increase in low-density lipoproteins (LDL) and cardiovascular risk (Eyres et al. 2016), there is still no consensus on the benefits of CO. The US Department of Agriculture (USDA, 2015), the World Health Organization (WHO, 2018) and the European Food Safety Authority (EFSA, 2017) recommend that daily intake of saturated fats not exceed 10% of the total calories or that it is as low as possible. In Brazil, the Brazilian Society of Endocrinology and Metabolism (SBEM) and the Association for the Study of Obesity and Metabolic Syndrome (ABESO) do not endorse the use of this product for weight loss and recommend its use in a restricted way (SBEM, ABESO 2015).

Thus, the present study evaluated the effects of coconut oil associated with a potentially acidifying diet offered to rats during the growing phase after weaning on acid-base status, renal function and metabolism.

Material and methods

The experimental procedures were performed in accordance with the guidelines of the Brazilian Society for Science in Laboratory Animals (SBCAL) and were approved by the Ethics Committee at the Federal University of Mato Grosso (protocol number: 23108.115132/2015-27).

Animals and diets

Male Wistar rats were obtained from the University's own breeding colony at weaning. At 30 days old, rats were randomly assigned to one of three different groups: SO, consisting of rats fed a control diet (AIN-93G); SO+CO, consisting of rats fed a diet containing a mix of soybean oil and coconut oil; and CO, consisting of the rats fed a diet containing coconut oil. The diets were isocaloric, as described in Table 1.

Table 1. Composition of experimental diets (g/kg)

Ingredients	**SO***	**SO+CO**	**CO**
Cornstarch	397.0	397.0	397.0
Casein (84% protein)	200.0	200.0	200.0
Dextrinized cornstarch	132.0	132.0	132.0
Sucrose	100.0	100.0	100.0
Soybean oil	70.0	35.0	-
Coconut oil	-	35.0	70.0
Fiber	50.0	50.0	50.0
AIN-93G mineral mix*	35.0	35.0	35.0
AIN-93G vitamin mix*	10.0	10.0	10.0
L-cystine	3.0	3.0	3.0
Choline chlorhydrate	2.5	2.5	2.5

*See Reeves et al. (1993) for more details

The fatty acid composition of the dietary lipids is reported in Table 2. The diets were prepared weekly and stored at -20°C. During the experimental period, the rats had access to their respective diets and to water ad libitum. Rats were maintained in collective cages (four rats/cage) and were housed at 22°C with a 12-h light–12-h dark cycle during the experimental period.

Table 2. Fatty acid composition of coconut oil and soybean oil

Fatty acids	SO*	CO**
Caproic C6:0	-	0.38
Caprylic C8:0	-	5.56
Capric C10:0	-	4.99
Lauric C12:0	-	45.78
Myristic C14:0	0.08	18.56
Palmitic C16:0	10.83	8.85
Stearic C18:0	3.36	3.39
Oleic C18:1	22.98	5.65
Linoleic C18:2	53.85	0.94

*Tabela Brasileira de Composição de Alimentos (TACO, 2011).

** Information taken from product packaging.

Experimental procedures

Food intake was recorded three times/week during the experimental period. The pre-weighed diet was provided, and after 48 h, rats were briefly removed from their cages. The amount of food remaining, including any food on the bottom of the cages, was recorded. Food intake was calculated as the weight (in grams) of diet provided less that recovered and expressed in absolute or relative values. To assess the relative food intake, the total food intake during the experimental period was normalised per 100 grams of body weight at 90 days of age. The measurement of body weight was performed once a week.

For urine collection, rats were maintained in metabolic cages overnight without diet and with free access to water, obtaining pure urine without contamination with faeces or feed. Measurements of pH and osmolality were performed using reagent strips (Uriclin 10, Laborclin Produtos para Laboratórios Ltda, Brazil). Urinary urea, creatinine, glucose and protein were measured by colourimetric methods using commercial kits (Wiener Lab Group, Brazil).

After seven weeks on the respective diets, measurements of glucose, ketone bodies, glycerol and lactate concentrations were performed after a 12-h fast or 2 h after overnight feeding. Then, rats were submitted to an oral glucose tolerance test (OGTT) followed by an insulin tolerance test (ITT). These three tests were performed at three-day intervals. At 90 days of age, rats were anaesthetised for gasometry and then euthanised by decapitation. Blood samples were collected, and serum was obtained by centrifugation to determine biochemical parameters**.** After a medium laparotomy, the retroperitoneal (RWAT), epididymal (EWAT) and perirenal (PWAT) white adipose tissues were removed, and the mass of the tissues was measured. The interscapular brown adipose tissue (IBAT) was removed, carefully cleaned to remove adhering fat and muscle, and weighed. The adiposity index was calculated as the sum of RWAT, EWAT and PWAT divided by body weight multiplied by 100.

The kidneys were removed, dissected, and weighed for morphometric analysis.

Measuring of blood glucose and ketone bodies, serum glycerol and plasma lactate

Blood, plasma or serum samples were obtained from the cut tip of the tail from rats fed and fasted (12 h) to determine glucose (Accu-Chek glucometer, Roche, Germany), ketone bodies (FreeStyleOptium Neo, Abbott, UK), lactate and glycerol (Bioclin/Quibasa, Brazil) concentrations.

OGTT

After a 6-h fast, glucose (200 g/L) was administered orally at a dose of 2 g/kg of body weight. Blood samples were obtained from the cut tip of the tail 0, 15, 30, 60, 90 and 120 min later for the determination of blood glucose using an Accu-Chek glucometer. The glucose response during the oral glucose tolerance test was calculated by estimating the total area under the glucose curve using the trapezoidal method (Matthews et al. 1990).

ITT

After a 6-h fast, insulin (regular) was administered intraperitoneally at a dose of 2.0 U/kg body weight. Blood samples were obtained from the cut tip of the tail at -5, 0, 5, 10, 15, 20 and 30 min post-injection for the determination of serum glucose concentrations. The glucose response during the insulin tolerance test was evaluated by the constant for blood glucose disappearance (K_{itt}), which was calculated from the slope of the fall in log-transformed plasma glucose after insulin administration (Lundbaek 1962), when the glucose concentration declined linearly.

Gasometry

After overnight fasting, rats were anaesthetised with an intraperitoneal injection of sodium thiopental (60 mg/kg), and whole blood was collected by cardiac puncture. Gasometry and haematologic parameters were measured with the i-STAT system (Abbott Laboratories, IL, USA) using an EC8+ cartridge according to the manufacturer's instructions.

Biochemical parameters

Serum total protein, albumin, creatinine, urea, AST (aspartate transaminase), ALT (alanine transaminase), Na^+, and K^+ were determined by enzymatic methods using commercial kits (Wiener Lab Group, Brasil). Serum osmolarity was calculated by the following formula: Calculated osmolarity = 2 (Na^+) + glucose + urea (all in mmol/L). The serum globulin concentration was obtained by subtracting the serum total protein from the serum albumin concentration value.

Serum triglycerides, total cholesterol, and high-density lipoprotein cholesterol (HDL) were analysed by an enzymatic method using commercial kits (Wiener Lab Group, Brasil). The values of VLDL and LDL were calculated according to the Friedewald equation:

VLDL= Triglycerides/5

LDL= Total cholesterol – (HDL + VLDL)

The atherogenic index was obtained using the following formula: atherogenic index= (total cholesterol-HDL)/HDL.

Because it was not possible to evaluate all variables in the same animal, the number of individual experiments varied among the groups.

Assessment of renal function

Glomerular filtration was assessed by creatinine clearance based on serum and urine creatinine levels, with values expressed in mL/min/kg BW, computed with the formula: Creatinine clearance = urine creatinine (mg/dL) x urine flow (mL/min) /serum creatinine (mg/dL)x BW(kg).

Urine flow was calculated by dividing 12 h of urine volume by 720, which corresponds to the number of minutes in 12 h (60 min x 12 h = 720): urine flow (mL/min) = value of urine volume (12 h)/720.

To calculate the fractional excretion of sodium, we used the values of the urine and serum sodium (mEq/L) and the urine and serum creatinine (mg/dL), using the formula: Fractional excretion of sodium (%)= (urine/serum sodium) / (urine/serum creatinine) X 100.

The values of the fractional excretion of potassium were obtained through the values of the urine and serum potassium (mEq/L) and the urine and serum creatinine (mg/dL), with the following formula: Fractional excretion of potassium (%)= (urine/serum potassium) / (urine/serum creatinine) X 100.

The protein/creatinine ratio (mg/dL) was obtained by dividing urine protein by urine creatinine at 12 h.The osmolar clearance was obtained by the formula: osmolar clearance= (urinary osmolality [mOsm/L] x urinary volume [ml/min]/serum osmolality [mOsm/L]) x body weight (kg). Water clearance was calculated by the following formula: Water clearance= osmolar clearance – urinary volume.

Histological processing

Four rats from each group were used for the histological studies. Kidney tissues were fixed in 4% paraformaldehyde solution for 24 h at 4°C, dehydrated in increasing concentrations of ethanol, clarified in xylol, and paraffin embedded using a histological processor (MTP 100 Slee, Mainz, Germany). Tissue was serially sectioned at 3-μm thickness throughout length using a microtome (Leica RM2125, Leica Biosystems Nussloch, Germany) and mounted on slides with an adhesive surface. The kidney sections were deparaffinised in an oven at 60°C for 2 h, followed by immersion in xylol

and descending concentrations of ethanol. Then, the slide was rehydrated and stained with haematoxylin and eosin for determination of glomerular and glomerular corpuscle areas using a light microscope at 5x magnification (Axio Scope. A1, Carl Zeiss, Oberkochen, Germany).

Statistical analysis

The results are expressed as the mean values with standard deviation for the number of rats indicated. Levene's test for the homogeneity of variances was initially used to determine whether the data complied with the assumptions necessary for a parametric ANOVA. When necessary, the data were log-transformed to correct for the variance in heterogeneity or non-normality (Sokal and Rohlf 1995). One-way ANOVA was used to compare the data from the SO, SO+CO and CO groups. When necessary, these analyses were complemented by the Tukey test to determine the significance of the individual differences. $P<0.05$ indicated statistical significance. All statistical analyses were conducted using the Statistic software package (Stat-soft).

Results

The initial and final body weights as well weight gain did not differ among groups. The total food intake was higher in the CO group than in the SO ($P<0.0001$) and SO+CO ($P<0.0003$) groups. However, the food intake relative to 100 g of body weight was similar in the CO and SO+CO groups; the CO group exhibited higher relative food intake than the SO group ($P<0.025$), whereas in the SO+CO and SO groups, this variable did not differ (Table 3).

Table 3. Initial and final body weight, body weight gain, total food intake and relative food intake from rats maintained on soybean oil (SO group), soybean oil + coconut oil (SO+CO) or coconut oil (CO) diets.

Variable	Group		
	SO (8)	SO+CO (8)	CO (8)
Initial body weight (g)	118± 27	144± 49	125± 30
Final body weight (g)	400± 48	390± 39	403± 59
Weight gain (g)	282± 45	248± 47	278± 72
Total food intake (g)	1233± 60[b]	1276± 62[b]	1445± 84[a]
Food intake (g/100g BW)	312± 38[b]	329± 28[ab]	363± 40[a]

Values are the mean with standard deviation for the number of rats shown in parentheses. Mean values with different superscript letters were significantly different ($P<0.05$, Tukey's test).

The post-prandial and fasting blood glucose (Figure 1A and 1E, respectively) and ketone bodies (Figure 1B and 1F, respectively) did not differ among groups. Plasma lactate concentrations in the post-prandial (Figure 1C) and fasting (Figure 1G) states were lower in the CO group than those in the SO group ($P<0.013$ and $P<0.024$, respectively), and those in the SO+CO group were similar to those in the SO and CO groups. The post-prandial serum glycerol concentration (Figure 1 D) was higher in the CO group than in both the SO group ($P<0.0005$) and the SO+CO group ($P<0.002$). Serum glycerol concentration in the fasting state did not differ among groups (Figure 1H).

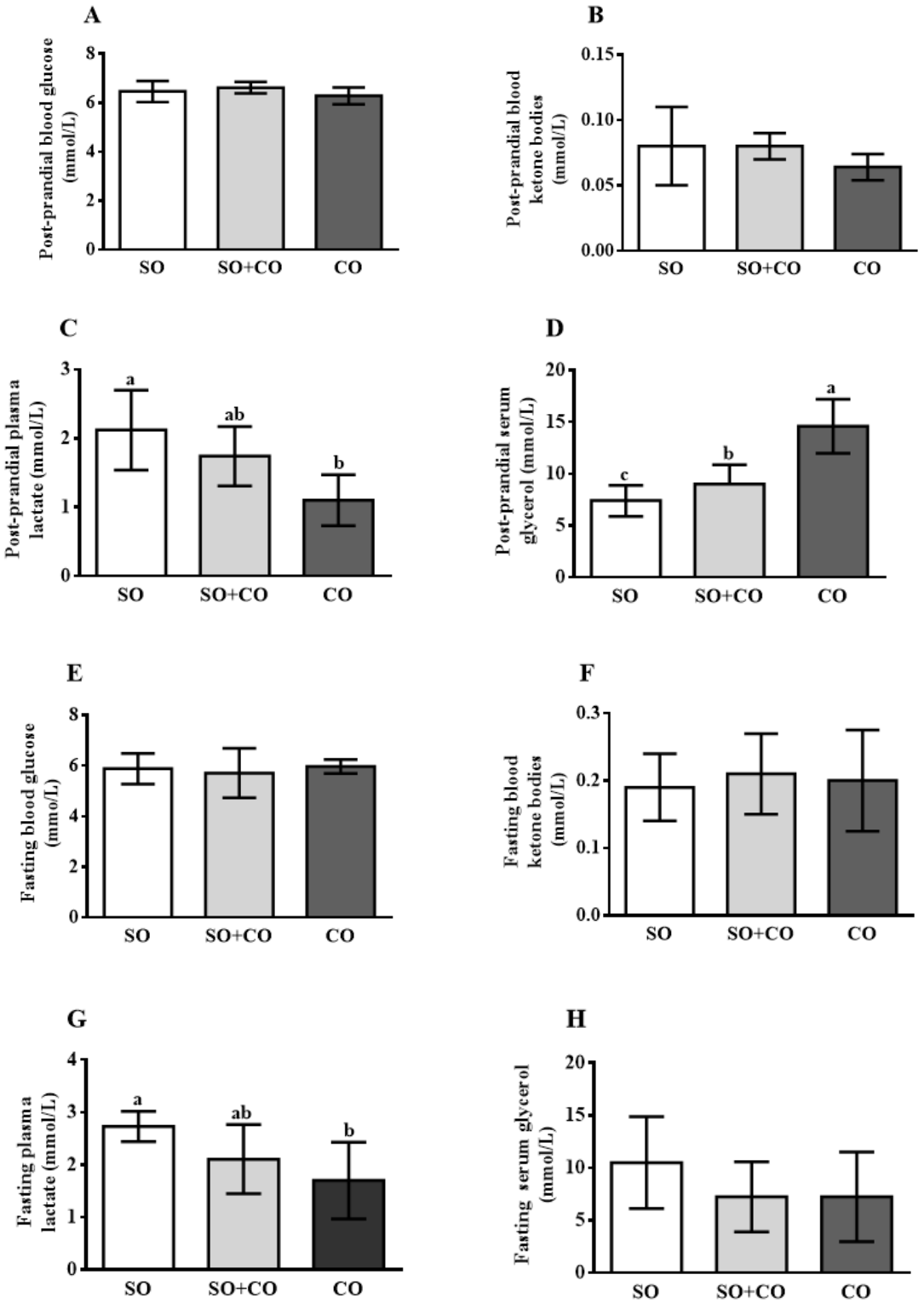

Figure 1. Post-prandial blood glucose (A) and ketone body (B), serum lactate (C) and glycerol (D) concentrations (n= 5 rats from each group); fasting blood glucose (E) and ketone body (F), serum lactate (G) and glycerol (H) concentrations from rats maintained on soybean oil (SO group), soybean oil + coconut oil (SO+CO) or coconut oil (CO) diets (n=6 rats from the SO group and n=8 rats from the SO+CO and CO groups)**.** Mean values with different superscript letters were significantly different (P<0.05, Tukey's test).

Gasometry parameters (pCO_2, HCO_3 and pH), haemoglobin and haematocrit did not differ among the three groups. The anion gap was lower in the CO group than in the SO group (P<0.0268), and in the SO+CO group, the anion gap did not differ from the SO and CO groups (Table 4).

Table 4. Haematological and blood gasometry parameters from rats maintained on soybean oil (SO group), soybean oil + coconut oil (SO+CO) or coconut oil (CO) diets

	Group		
Variable	SO (5)	SO+CO (6)	CO (6)
Haemoglobin (g/dl)	15.5± 0.3	15.8± 0.5	16.0± 0.6
Haematocrit (%)	45.6± 0.9	46.7± 1.4	47.0± 1.8
pCO_2 (mmHg)	73± 20	68± 4	57±6
HCO_3 (mmol/L)	32.4± 2.0	35.4± 1.5	36.1± 5.0
pH	7.27± 0.13	7.32± 0.04	7.39±0.06
Anion gap (mEq/L)	10.8±1.5[a]	8.5±2.2[ab]	7.5±1.5[b]

Values are the mean with standard deviation for the number of rats shown in parentheses. Mean values with different superscript letters were significantly different (P<0.05, Tukey's test).

The cumulative water intake was higher in the CO group than in the SO group (P<0.034). In the SO+CO group, this variable did not differ from the CO group or the SO group. Urine volume, flow, density, pH and osmolality were similar in the groups (Table 5).

Table 5. Urinary parameters from rats maintained on soybean oil (SO group), soybean oil + coconut oil (SO+CO) or coconut oil (CO) diets

Variable	Group		
	SO (8)	SO+CO (8)	CO (8)
Cumulative water intake (ml)	1454± 222[b]	1450± 49[ab]	1726± 30[a]
Urine volume (ml/12h)	3.84± 2.08	3.09±1.90	3.46± 1.25
Urine flow (ml/min)	0.005± 0.003	0.004±0.003	0.0063± 0.004
Urine density	1023± 8	1027± 3	1024±8
Urine pH	6.6± 0.2	6.6± 0.3	6.8± 0.3
Urine osmolality (mmol/L)	763± 271	875± 106	784± 261

Values are the mean with standard deviation for the number of rats shown in parentheses. Mean values with different superscript letters were significantly different (P<0.05, Tukey's test)

The urinary sodium, potassium, glucose, protein, creatinine and protein/creatinine ratio were similar in all groups. Urinary urea was higher in the CO group than in the SO group (P<0.042), and in the SO+CO group, urea was similar to that in the SO and CO groups (Table 6).

Table 6. Urinary electrolytes and metabolites from rats maintained on soybean oil (SO group), soybean oil + coconut oil (SO+CO) or coconut oil (CO) diets

	Group		
Variable	SO	SO+CO	CO
Na^+ (mmol/L)	46± 27 (7)	51± 14 (8)	67± 24 (7)
K^+ (mmol/L)	38± 30 (8)	47±17 (8)	46± 27 (8)
Glucose (mmol/L)	2.9± 2.0 (7)	2.4±0.8 (8)	2.6± 1.6 (7)
Protein (mg/dl)	6.5± 2.7 (8)	7.4± 1.6 (8)	8.9±3.1 (8)
Creatinine (mg/dl)	146± 110 (8)	213± 63 (8)	152± 67 (8)
Protein/creatinine ratio	0.66± 0.32 (7)	0.44± 0.12 (8)	0.87± 0.61 (8)
Urea (mg/dl)	2519±1706[b] (8)	4594±1359[ab] (8)	4725±1971[a] (8)

Values are the mean with standard deviation for the number of rats shown in parentheses. Mean values with different superscript letters were significantly different (P<0.05, Tukey's test).

Osmolar (Figure 2B) and water (Figure 2C) clearances were higher in the CO group than in the SO group (P<0.025 and P<0.0193, respectively), but both groups were similar to the SO+CO group. The creatinine clearance (Figure 2D) was higher in the CO (P<0.027) and SO+CO (P<0.0051) groups than that in the SO group. Fractional excretion of sodium (Figure 2E) was lower in the SO+CO group than in the SO group (P<0.0386), and this variable did not differ between the CO and SO groups. The serum osmolarity (Figure 2A) and the fractional excretion of potassium (Figure 2F) were similar in the three groups.

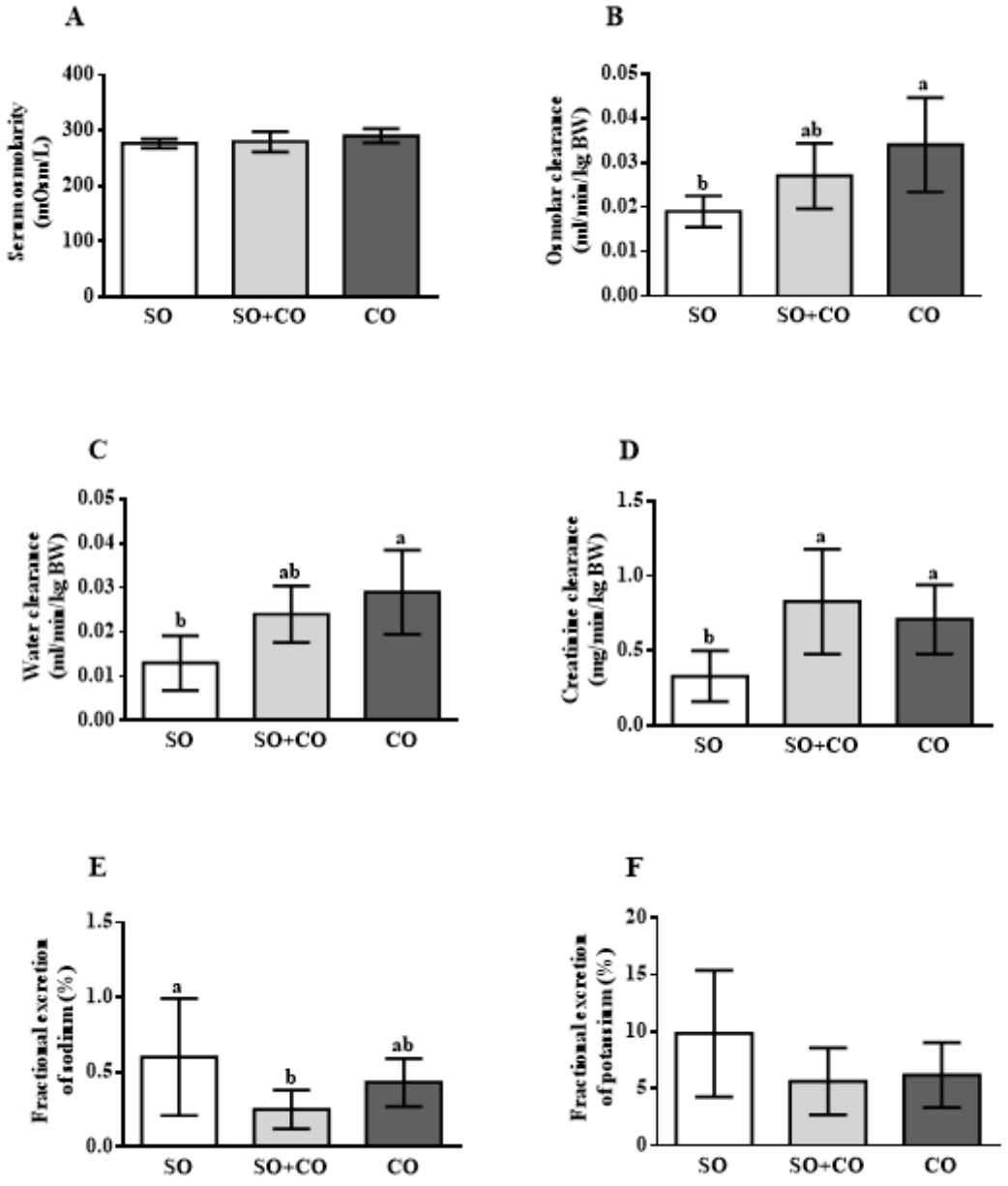

Figure 2. Serum osmolarity (A), osmolar clearance (B), water clearance (C), creatinine clearance (D), fractional excretion of sodium (E) and fractional excretion of potassium (F) from rats maintained on soybean oil (SO group), soybean oil + coconut oil (SO+CO) or coconut oil (CO) diets. Mean values with different superscript letters were significantly different (P<0.05, Tukey's test).

Kidney weight (g and g/100 g body weight) (Figures 3A and 3B) did not differ among the groups. The glomerular area (Figure 3C) and glomerular corpuscle area (Figure 3D) were similar between the CO and SO+CO groups, and in both groups, these variables were higher in comparison to the SO group (P<0.05).

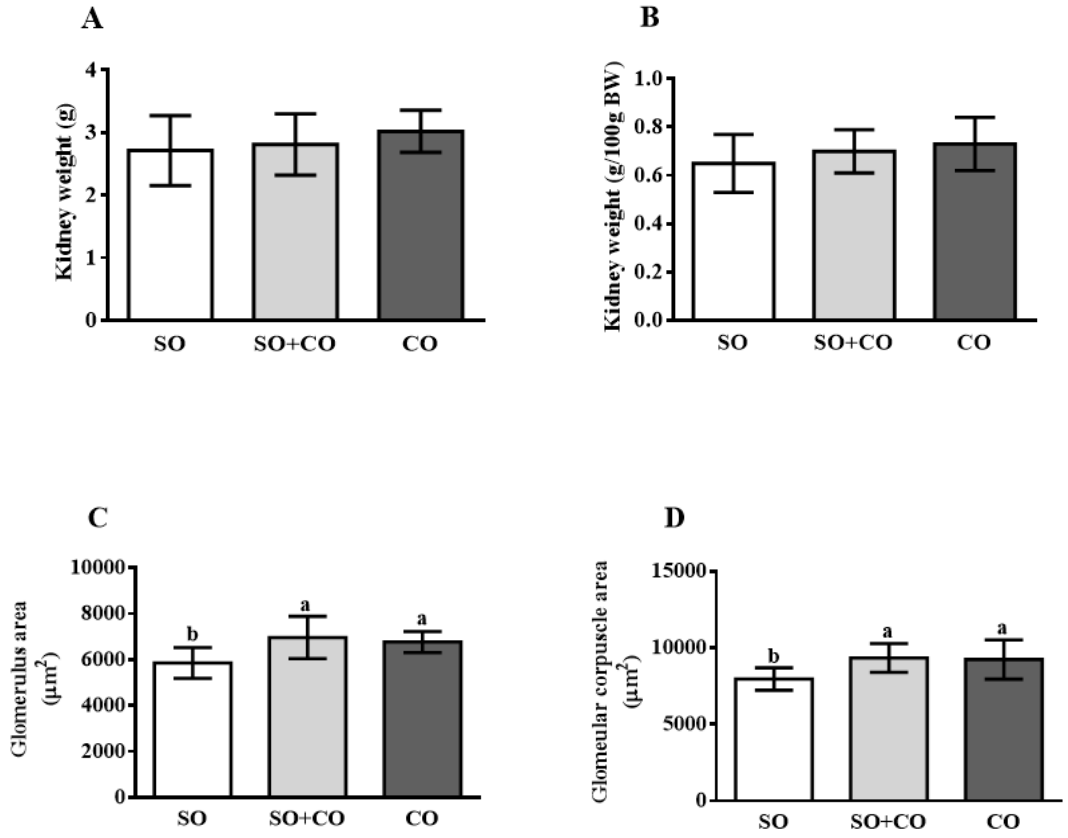

Figure 3. Kidney weight in absolute (A) and relative to 100 g of body weight (B) values, glomerulus area (C) and glomerular corpuscle area from rats maintained on soybean oil (SO group), soybean oil + coconut oil (SO+CO) or coconut oil (CO) diets Mean values with different superscript letters were significantly different ($P<0.05$, Tukey's test).

Serum total protein (Figure 4A) and globulin (Figure 4C) concentrations were higher in the CO group than in the SO group ($P<0.0328$ and $P<0.0116$); the SO+CO group did not differ from the CO or SO group. Serum albumin concentrations (Figure 4B) were lower in the SO+CO group than in the SO ($P<0.0017$) and CO ($P<0.040$) groups, whereas serum ALT levels (Figure 4E) in the SO+CO and CO groups were similar, and both were lower compared to the SO group ($P<0.0011$ and $P<0.0056$). The serum AST level (Figure 4D) was lower in the SO+CO group than that in the SO group ($P<0.012$), and the CO group did not differ from the other groups.

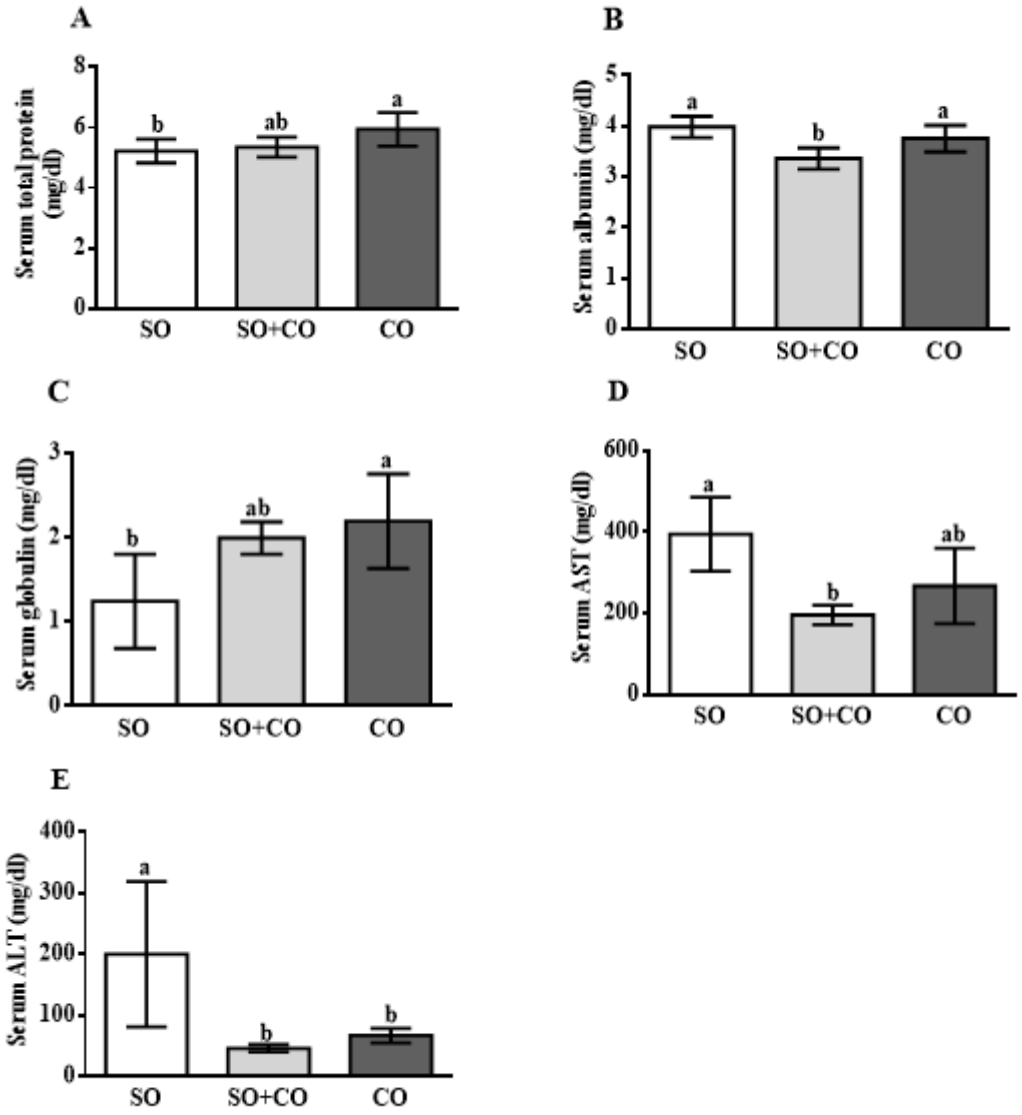

Figure 4. Serum total protein (A), albumin (B), globulin (C), AST (D) and ALT (E) concentrations from rats maintained on soybean oil (SO group), soybean oil + coconut oil (SO+CO) or coconut oil (CO) diets. Mean values with different superscript letters were significantly different ($P<0.05$, Tukey's test).

The constant for plasma glucose disappearance (K_{itt}) was lower in the SO+CO group than that in the CO ($P<0.0278$) and SO ($P<0.0260$) groups (Figure 5A). The area under the glucose curve during the OGTT did not differ among the groups (Figure 5B).

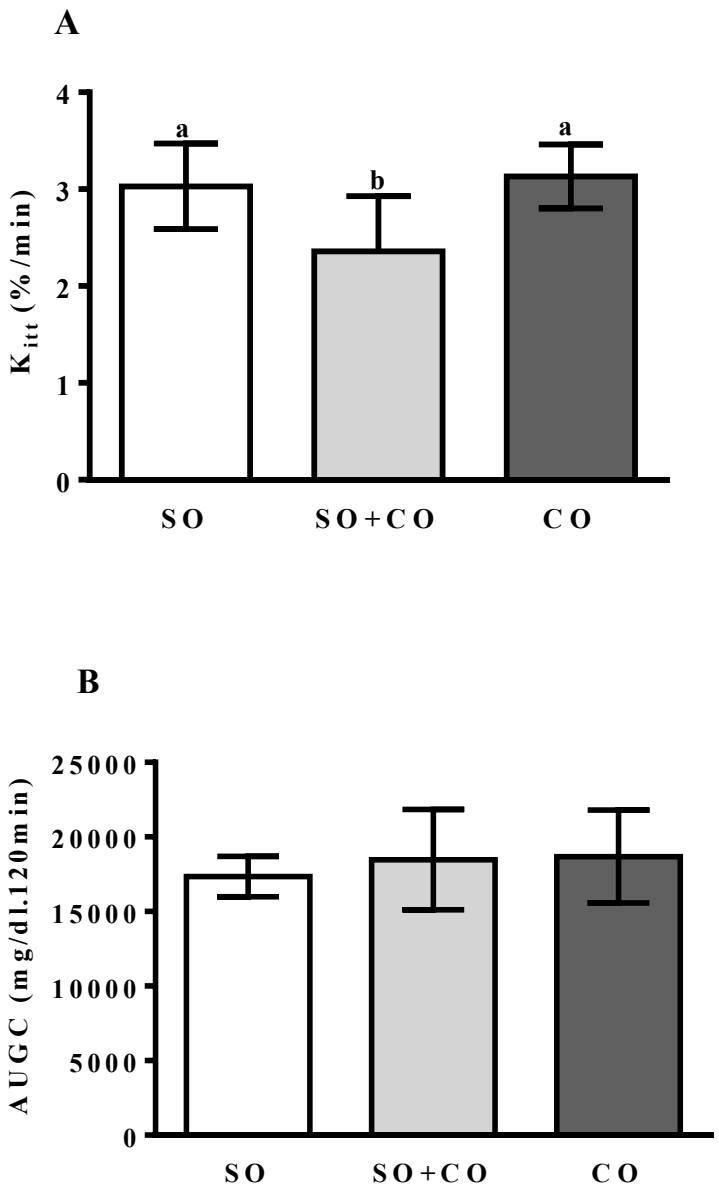

Figure 5. Glucose disappearance ratio (K_{itt}) obtained from the intraperitoneal insulin tolerance test (A) and area under the glucose curve obtained from the oral glucose tolerance test (B) from rats maintained on soybean oil (SO group), soybean oil + coconut oil (SO+CO) or coconut oil (CO) diets. Mean values with different superscript letters were significantly different (P<0.05, Tukey's test).

The weights of IBAT, RWAT and PWAT in g and g/100 g of body weight, as well as the adiposity index, were similar in all groups. The EWAT weight in g and g/100 g of body weight was higher in the CO group than in the SO group (P<0.031), and these variables in the SO+CO group were similar to those in both the SO and CO groups (Table 7).

Table 7. Weight of white and brown adipose tissues from rats maintained on soybean oil (SO group), soybean oil + coconut oil (SO+CO) or coconut oil (CO) diets

Variable	Group		
	SO (8)	SO+CO (8)	CO (8)
IBAT			
(g)	0.39±0.10	0.29±0.06	0.39±0.10
(g/100g BW)	0.09± 0.02	0.07±0.02	0.09±0.01
RWAT			
(g)	6.44± 2.41	6.41±1.71	7.25±2.55
(g/100g BW)	1.55±0.51	1.61± 0.39	1.70±0.37
PWAT			
(g)	0.80± 0.44	0.66±0.37	0.87±0.34
(g/100g BW)	0.19±0.11	0.16±0.08	0.21±0.07
EWAT			
(g)	4.28 ± 2.69^{b}	6.20 ± 1.43^{ab}	8.00 ± 3.55^{a}
(g/100g BW)	1.03 ± 0.63^{b}	1.56 ± 0.33^{ab}	1.91 ± 0.75^{a}
Adiposity index	2.75±0.97	3.33±0.70	3.83±0.99

Values are the mean with standard deviation for the number of rats shown in parentheses. Mean values with different superscript letters were significantly different ($P<0.05$, Tukey's test). IBAT: interscapular brown adipose tissue; RWAT: retroperitoneal white adipose tissue; PWAT: perirenal white adipose tissue; EWAT: epididymal white adipose tissue.

Serum triglyceride (Figure 6A), VLDL (Figure 6C) and HDL (Figure 6E) concentrations were higher in the CO group than concentrations in the SO+CO ($P<0.0081$, $P<0.0054$, and $P<0.0003$, respectively) and SO groups ($P<0.0061$, $P<0.0038$, and $P<0.0003$, respectively). The serum total cholesterol concentration (Figure 6B) was similar in the SO+CO and CO groups, and both were higher compared to the SO group ($P<0.00014$ and $P<0.00016$, respectively). The serum LDL level

(Figure 6D) and atherogenic index (Figure 6F) were higher in the SO+CO group than in the CO (P<0.00018) and SO groups (P<0.00018).

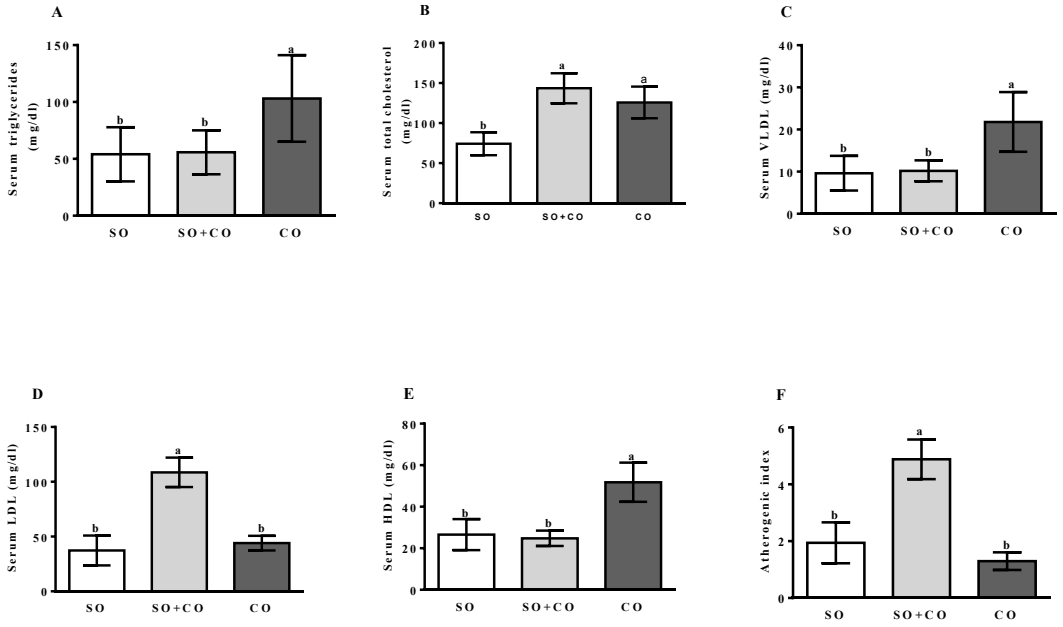

Figure 6. Serum triglycerides (A), total cholesterol (B), VLDL (C), LDL (D), HDL (E) and the atherogenic index (F) of rats maintained on soybean oil (SO group), soybean oil + coconut oil (SO+CO) or coconut oil (CO) diets. Mean values with different superscript letters were significantly different (P<0.05, Tukey's test).

Discussion

The present study evaluated the effects of CO associated with a potentially acidifying diet offered to rats during the growing phase after weaning on acid-base status, renal function and metabolic profile. In agreement with other studies (LaBarrie and St-Onge 2017, Kinsella et al. 2017), CO did not promote satiety in our animals; on the contrary, it increased food intake but did not change body weight. The increased food intake associated with unaltered body weight suggests a thermogenic effect of CO, already shown by studies that evaluated the effect of MCT on diet-induced thermogenesis (Alexandrou et al. 2007; Kasai et al. 2002).

The ketogenic effect attributed to MCFAs was not observed in our rats, possibly because unlike MCT oil, which produces a large rise in plasma ketones, ingestion of CO

(in which lauric acid is the main MCFA) leads to a delayed and less prominent rise in ketone bodies (McCarty and Di Nicolantonio 2016). Evaluating the elevated post-prandial serum glycerol levels observed in both the SO+CO and CO groups (but to the highest degree in the latter), it is unlikely that the ketogenic effect of these diets, such as ketone bodies, exerts anti-lipolytic actions on adipose tissue (Robinson and Williamson 1980). However, reduced plasma lactate levels (the end product of glycolysis) observed in rats maintained on a CO diet weakens that hypothesis, since ketone bodies attenuate glucose utilisation in peripheral tissues (Robinson and Williamson 1980). Notably, exacerbated post-prandial lipolysis (assessed by serum glycerol levels) was observed in insulin-sensitive animals (CO group), and middle lipolysis was observed in insulin-resistant animals (SO+CO group). These inconsistencies deserve further study to elucidate their causes.

Although the diets used in this study are presumably acidifying because they contain animal protein (casein) and coconut oil in their composition, the blood gas analysis did not indicate metabolic acidosis. The profile presented by all groups (pH < 7.4, PCO_2 > 24 mmHg, HCO_3> 24 mmol/L) was compatible with respiratory acidosis, probably triggered by anaesthesia (Kaczmarczyk and Reinhardt, 1975). Notably, rats maintained with CO exhibited a reduced anion gap, which could be attributed to at least three factors: 1) increased serum globulin levels, as observed here; 2) hypercalcaemia; and 3) hypermagnesaemia (Lee et al. 2006). Hyperglobulinaemia is known to occur in situations of inflammation (alpha globulinaemia) or disorders of lipid metabolism (beta globulinaemia) (Larson 1974). Hypercalcaemia is observed in metabolic acidosis due to increased calcium efflux from bone (Lemann et al. 1966; Lemann et al. 1967) and stimulation of parathyroid hormone (PTH) secretion (Bichara et al. 1990; Lopez et al. 2002; Graham et al. 1997).

Interestingly, in the present study, CO reduced serum ALT levels, an indicator of haemodilution (Sette and Lopes 2014), which in our rats fed CO was corroborated by elevated cumulative water intake and osmolar and water clearances. The unaltered serum osmolarity observed in these rats did not invalidate this hypothesis, as this variable is a poor predictor of changes in hydration status when a single, fasted morning blood sample is collected (Armstrong et al. 2013). The haemodilution observed in these rats can contribute to decreased oxygen carrying capacity, decreased plasma colloid osmotic pressure, and elevated interstitial water content (English et al. 1971).

Our animals fed CO or SO+CO exhibited high creatinine clearance and increased glomerular and glomerular corpuscle areas. Moreover, rats maintained with CO displayed high urinary urea. It has been shown that in response to metabolic acidosis, significant functional changes take place in the kidney, including an increase in renal plasma flow and glomerular filtration rate, which probably serve to remove the excess acid load (Györke et al. 1991; Sartorius et al. 1949; Sulyok et al. 1990; Garibotto et al. 2009).. Increased urinary urea seems to be an attempt to excrete the acid load since the kidney net acid excretion rate varies directly with the urinary urea excretion rate (Lemann 1999). The reduced fractional excretion of sodium observed in our SO+CO rats is also an indication of metabolic acidosis. Chloride (and sodium) urinary loss induces negative sodium chloride balance, with secondary activation of the renin-angiotensin-aldosterone system and a subsequent rise in plasma and urine aldosterone concentrations (Györke et al. 1991).

Finally, the expected negative effect of insulin sensitivity triggered by metabolic acidosis (DeFronzo and Beckles 1979) was observed only in the SO+CO group but was not enough to declare glucose intolerance. The most significant metabolic alteration observed in rats maintained on CO or SO+CO diets was in the serum lipid profile and

increased EWAT weight. The latter alteration was not surprising because the reducing effect of adiposity has been associated with MCT oil consumption and not CO (St-Onge and Jones 2003). As expected (McCarty and DiNicolantonio, 2016), CO produced a higher impact on serum HDL levels that resulted in an increase in total serum cholesterol but a reduced ratio of total cholesterol to HDL cholesterol. Moreover, CO increased serum triglycerides and VLDL levels. The high triglycerides and VLDL levels are attributed to lauric acid, which has a higher propensity to be absorbed via the lymphatics (presumably reflecting its greater capacity for incorporation into triglycerides); therefore, its access to the liver is delayed (Sigalet et al. 1997; Sigalet and Martin 1999). In contrast, the SO+CO groups displayed high serum LDL levels and atherogenic indices. However, it has been shown that the LDL particles raised by the consumption of CO are large and buoyant, which are less related to coronary diseases when compared with small and dense LDL particles (Katan et al. 1994).

Taking into consideration the results obtained in the present study, we conclude that the administration of a casein-based diet containing coconut oil during weaning growth did not alter the acid-base state but impaired renal structure and function and contributed to an atherogenic profile in adulthood.

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Menezes as a partial requirement for a Master's degree in Nutrition, Food and Metabolism at the College of Nutrition, UFMT.

Compering Interesting

None declared.

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ANEXOS

Anexo 1: Documento de aprovação no Comitê de Ética no Uso de Animais

UNIVERSIDADE FEDERAL DE MATO GROSSO
COMITÊ DE ÉTICA NO USO DE ANIMAIS

CEUA
COMITÊ DE ÉTICA NO USO DE ANIMAIS

CERTIFICADO

Certificamos que o Protocolo Nº 23108.115132/2015-27, sobre "Efeito do consumo de óleo de coco extra virgem sobre o perfil somático e lipídico de ratos machos adultos", sob a responsabilidade de **Profª Drª MÁRCIA QUEIROZ LATORRACA**/Caroline Corrêa Menezes & Col., está de acordo com os Princípios Éticos na Experimentação Animal adotados pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), tendo sido aprovado pelo Comitê de Ética no Uso de Animais (CEUA)-UFMT em reunião ordinária de **10/12/2015**.

CERTIFICATE

We certify that the protocol Nº 23108.115132/2015-27, entitled "Effect of extra virgin coconut oil consumption on somatic and lipid profile of adult male rat", is in agreement with the Ethical Principles for Animal Research established by the National Council for Control of Animal Experimentation (CONCEA). This project was approved by the institutional Committee for Ethics in the Use of Animals (Federal University of Mato Grosso – UFMT) on **Dec 10, 2015**.

Cuiabá-MT, 11 de dezembro de 2015.

Prof. Dr. Roberto Vilela Veloso
Presidente

Prof. Dr. Daniel Moura de Aguiar
Vice-Presidente

Universidade Federal de Mato Grosso – UFMT
Cidade Universitária – Av. Fernando Correa da Costa, 2.367
Bairro Boa Esperança – CEP 78060-900 – CUIABÁ-MT, Brasil.

Telefone: (65) 3615 8829
Fax.: (65) 3615 8254
E-mail: cepa@ufmt.br