MATHEUS SOARES DA SILVA FERREIRA

RACTOPAMINA EM PROGRAMAS *STEP UP* ASSOCIADO A DOIS NÍVEIS DE LISINA EM RAÇÕES PARA SUÍNOS EM TERMINAÇÃO

LAVRAS – MG

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Ficha Catalográfica Elaborada pela Coordenadoria de Produtos e Serviços da Biblioteca Universitária da UFLA

Ferreira, Matheus Soares da Silva.
Uso da ractopamina em programa step up associada a dois níveis de lisina em rações para suínos em terminação / Matheus Soares da Silva Ferreira. – Lavras : UFLA, 2014.
100 p. : il.

Tese (doutorado) – Universidade Federal de Lavras, 2014.
Orientador: Raimundo Vicente de Sousa.
Bibliografia.

1. Machos castrados. 2. Agonista β-adrenérgico. 3. Síntese proteica. 4. mTOR. 5. Perfil de ácidos graxos. I. Universidade Federal de Lavras. II. Título.

CDD – 636.4085

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APROVADA em 17 de fevereiro de 2014.

Dr. Vinícius de Souza Cantarelli	UFLA
Dr. Márcio Gilberto Zangeronimo	UFLA
Dr. Hunaldo Oliveira Silva	UFS
Dr. Allan Paul Schinckel	PURDUE UNIVERSITY

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AGRADECIMENTOS

À Universidade Federal de Lavras (UFLA) e ao Programa de Pós-graduação em Ciências Veterinárias do DMV/UFLA, pela oportunidade de cursar o doutorado em tão distinta intituição;

À Capes, pela concessão de bolsa de estudos no Brasil, bem como pela concessão de bolsa para Doutorado sanduíche no exterior;

Ao professor, e acima de tudo amigo, Dr. Raimundo Vicente de Sousa, pela confiança e orientação no trabalho que resultou nesta tese, pelos inúmeros ensinamentos e conselhos que me guiaram durante toda minha pós-graduação.

Ao professor Dr. Vinícius de Souza Cantarelli, pela amizade e pela real e valorosa coorientação;

Ao funcionário do DMV William César Cortez, do laboratório de Fisiologia e Farmacologia, por toda a presteza nos momentos necessários e pelo sempre bom papo;

Ao funcionário da Centro de Experimentação em Suinocultura do DZO/UFLA, Hélio Rodrigues, e à toda equipe do Núcleo de Estudos em Suinocultura NESUI, sem os quais a condução do experimento seria impossível.

À *Purdue University* - E U. A., em especial ao professor Dr. Allan Paul Schinckel do *Animal Sciences Departament*, por aceitar ser meu orientador estrangeiro durante o Doutorado Sanduíche, permitindo-me a oportunidade de conhecer e conviver o dia a dia de uma instituição conhecida mundialmente por suas pesquisas, além da chance de ter contato com novas culturas e pessoas. Agradeço também por ter aceitado o convite para vir ao Brasil participar da banca de avaliação desta tese. *Thank you Dr. Schinckel, for the great friendship and taughts. I always behave myself!*

Aos professores; Dr. Márcio Gilberto Zangeronimo e Dr. Hunaldo Oliveira Silva, por aceitar o convite em participar da banca avaliadora;

Aos colegas de Pós-Graduação, pelos inúmeros momentos de risadas, convívio, apoio e amizade. Em especial à Vivian de Oliveira Silva, Ana Cláudia Alves, Letícia Mendonça e Thiago dos Santos Araújo, companheiros de bancada de muitos momentos, e aos casais Bob & Andressa e Ana & Gus, pelos momentos de descontração;

Aos diversos amigos fconquistados nos E.U.A. e no Brasil que compartilharam agradáveis momentos no exterior: Shigeru & Emiko Hayashida, Matt Asmus, Zach Rambo, Aaron Jones, Ozana Zacaroni, César Garbossa, Hebert Silveira, Diego Brandão, Igor & Gabriela, Márcio & Aline, Ankur & Jenette, *thank you guys for such great moments*.

Meus mais profundos agradecimentos vão para àqueles que são a minha razão de viver e lutar todos os dias; a minha famíla. Mayesse e Larissa, minhas joias mais preciosas, obrigado por estarem ao meu lado sempre, pelo carinho e paciência;

Pai, José Antônio Ferreira, e mães (Alcione Turchetti e Thelma Antun Ferreira), por terem me ensinado tudo o que sei sobre viver, lição mais valiosa e que não se aprende em livros;

Meus sogros, Dona Cida e seu Rodrigues, por todo o carinho;

Lucas Ferreira, meu irmão, pela amizade, suporte e companheirismo; Lilian e Rogério, meus cunhados, pelos ótimos momentos que desfrutamos juntos e pelos churrascos que continuaremos a fazer;

Muito obrigado!

"O último esforço da razão é reconhecer que existe uma infinidade de coisas que a ultrapassam."

"La dernière démarche de la raison, c'est de reconnaître qu'il y a une infinité de choses qui la surpassent."

Blaise Pascal

RESUMO GERAL

Foi conduzido um estudo para avaliar os efeitos da ractopamina (RAC) associada a dois níveis de lisina em rações para suínos em terminação. Cento e oito suínos com peso inicial de 75,37 ± 2,88 foram alocados entre nove tratamentos: controle negativo, sem adição de RAC e com níveis normais de lisina acima dos níveis basais (NC); dose constante de 7,5 ppm de RAC por 28 dias; 5 ppm de RAC por 14 dias, seguidos de 10 ppm por 14 dias (*step up* 1); 5 ppm de RAC por 21 dias, seguidos por 10 ppm por 7 dias (*step up* 2); e 5 ppm de RAC por 7 dias, seguidos por 10 ppm por 21 dias (*step up* 3). Os tratamentos com RAC foram testados com 15 ou 30% a mais de lisina acima dos requerimentos basais, resultando em esquema fatorial 4x2+1, com seis repetições. A RAC melhorou ($P < 0,05$) a eficiência alimentar (EA) e a eficiência na utilização de energia (EE) na primeira metade do experimento, e o ganho de peso (GP) na segunda metade em relação ao controle negativo (NC) ($P < 0,05$). Nas duas últimas semanas do experimento observou-se efeito positivo da RAC no GP, GPMD, EA e EE ($P < 0,01$). Considerando o período experimental completo, animais suplementados com RAC apresentaram maiores GP e GPMD ($P < 0,05$). Foram observados incremento ($P < 0,05$) de 23% na EA e 30% na EE em animais que receberam RAC ($P < 0,05$). PC e AOL foram maiores ($P < 0,01$) nos animais dos grupos com RAC. Os *western blots* mostraram efeito positivo da RAC na ativação da via da mTOR, uma vez que a concentração de p-$P70^{S6K}$ em células musculares foi maior para amostras de animais que receberam RAC e 15% de lisina em comparação com NC ($P < 0,10$). Os programas com 15% de lisina reduziram ($P < 0,01$) a concentração de HDL-c, enquanto que a RAC provocou aumento na atividade da AST ($P = 0,01$). O *step up* 2 com 15% de lisina foi 14,65% superior ao *step up* 2 com 30% de lisina ($P < 0,05$) para os MUFA. Conclui-se que a RAC foi efetiva na promoção da eficiência da produção, bem como que a adição de 15% de lisina foi suficiente para a ótima performance dos suínos neste estudo, entretanto os programas de *step up*não sobrepuseram os programas de suplementação da RAC em níveis constantes. Os resultados deste estudo sugerem que a estimulação da síntese proteica decorrente do uso da RAC ocorre por meio da ativação da via celular da mTOR. Os parâmetros bioquímicos do sangue e a qualidade da carne não foram afetados pela RAC. O perfil lipídico da carne não foi alterado. Ademais, a adição de 15% de lisina acima do requerimento basal na ração permitiu aos suínos responder de uma maneira semelhante à adição de 30%.

Palavras-chave: Machos castrados. Agonista β-adrenérgico. Síntese proteica. mTOR. Perfil de ácidos graxos.

GENERAL ABSTRACT

A study was conducted to evaluate the effects of ractopamine (RAC) associated to two additional levels of lysine fed to finishing pigs. One hundred-eight pigs (initially 75,37 kg ± 2.88) were allotted to one of the nine treatments: negative control without addition of RAC nor lysine (NC), 7.5 ppm RAC constantly, 5 ppm RAC for 14 days followed by 10 ppm for 14 days (step up 1), 5 ppm RAC for 21 days followed by 10 ppm for 7 days (step up 2) and 5 ppm RAC for 7 days followed by 10 ppm for 21 days (step up 3). On constant and step up treatments were added 15 or 30% lysine above basal requirements resulting on a 4x2+1 factorial with six replicates. RAC fed animals had better ($P < 0,05$) GF and energy efficiency of utilization (EF) in the first half of the trial, and greater ($P < 0,05$) ADG on the second half. On the second half of the trial RAC had a positive effect ($P < 0,01$) on weight gain, ADG, GF and EF. Considering the overall period RAC-treated pigs had greater BW and ADG ($P < 0,05$). An average of 23% improvement ($P < 0,05$) on feed efficiency and 30% improvement on energy efficiency of utilization were observed for RAC-fed pigs. Chilled carcass weight and loin eye area were improved ($P < 0,01$) for RAC-fed animals. Western blots showed a positive effect of RAC on the activation of the mTOR pathway, once a higher amount of the p-$P70^{S6K}$ on muscle samples of RAC-fed animals with 15% additional lysine compared to NC ($P < 0,10$) was found. Programs with 15% additional lysine decreased ($P < 0,01$) the concentration of serum HDL-c, while RAC had a significant effect on increasing AST ($P = 0,01$). Step up 2 with 15% lysine was 14,65% higher than step up 2 with 30% lysine for MUFA ($P < 0,05$). It can be concluded that RAC was effective on improving efficiency of production, as well as, lysine supplementation of 15% was enough for optimal performance of the pigs in this study, however, step up programs did not overcome RAC constant programs. Results in this study suggest that RAC stimulation of protein synthesis occurs through mTOR signaling pathway. Blood parameters and meat quality were not affected by RAC, thus keeping standard quality. Lipid profile of the meat was not changed. Moreover, 15% additional lysine above basal levels rations allowed the pigs to respond in a similar fashion to the 30% additional lysine ration, therefore, by using 15% additional lysine amino acids might be saved on formulation of the feed.

Keywords: Barrows. β-agonist. Lysine. Protein synthesis. mTOR. Fatty acid profile.

SUMÁRIO

PRIMEIRA PARTE

1 INTRODUÇÃO

Em 2013, dados do *Population Reference Bureau* (POPULATION REFERENCE BUREAU, PRB, 2013) mostraram que a população mundial era de 7,1 bilhões de pessoas, e estimada em 9,7 bilhões para o ano de 2050. Foi estimada para o continente africano uma população 2,2 vezes maior que a população em 2013, enquanto que a média de crescimento da população mundial é 1,4 vezes. No ano de 2011, 426 milhões de pessoas viviam nas 30 maiores áreas metropolitanas do planeta, ao passo que em 1950 eram 117 milhões (PRB, 2013). Esses números demonstram a dimensão do impacto que o aumento populacional no planeta Terra causará na produção de alimentos e na chamada sustentabilidade. Em uma matemática simples chega-se à conclusão que, com mais pessoas vivendo no planeta, a quantidade de alimento a ser produzida para sustentar a população em 2050 necessitará ser maior que a quantidade hoje produzida. Ressalta-se ainda que em 2013/2014 o meio ambiente, de uma forma geral, está mais degradado que em 1950, e que provavelmente estaria ainda mais degradado se o planeta estivesse produzindo alimentos para 9,7 bilhões de pessoas.

Para que no futuro seja possível maior produção de alimentos e menor dano ambiental, o uso de técnicas para o aumento da produtividade de cada unidade produtora é altamente desejável. Nessa ótica, especialmente as carnes e produtos de origem animal são hoje foco de muitos estudos.

Segundo a Organização para a Agricultura e Alimentação da Organização das Nações Unidas (FAO) existe uma tendência sobre a economia global alimentícia de forma que vem sendo observada uma alteração dos hábitos de consumo em direção ao aumento do consumo de produtos animais. Soma-se a

isso as implicações ambientais associadas à atual produção e à expansão da produção, de produtos de origem animal, principalmente desmatamentos e emissão de efluentes poluentes (FAO, 2003). Os governos dos países exportadores e a indústria farmacêutica veterinária têm importante papel no alcance destes objetivos, principalmente por meio do desenvolvimento de tecnologias que proporcionam melhorias na eficiência de produção dos animais. Desta forma seriam atendidos os anseios dos produtores, que teriam maior rentabilidade na sua atividade, dos consumidores finais, os quais são exigentes no que diz respeito ao acesso a produtos de alta qualidade, bem como quanto ao sabor e a manutenção das características nutricionais da carne comprada no comércio varejista, e também daqueles que se esforçam para o desenvolvimento de atividades que melhor atendam o conceito de sustentabilidade. A carne suína está diretamente ligada nesse contexto uma vez que é a mais consumida fonte de proteína animal no mundo (UNITED STATES OF AMERICA, USA, 2012) e a previsão é que continue sendo até 2015 (FAO, 2003).

Um aditivo alimentar, o qual tem por finalidade o aumento da eficiência de produção da carne suína, é a ractopamina (RAC) (CARR et al., 2008). Esta substância é adicionada à ração de suínos em terminação e promove uma série de alterações metabólicas que culminam com o aumento do peso corpóreo do suíno por meio da menor deposição de tecido adiposo na carcaça (MIMBS et al., 2005), porém, com aumento da massa magra, isto é, da musculatura (BOHRER et al., 2013).

Quando se fala em aumento muscular, que fundamentalmente trata-se de aumento da síntese proteica no tecido muscular, o principal aminoácido a ser considerado é a lisina. A adoção de níveis de lisina precisos para atender o requerimento do animal em resposta ao fornecimento da RAC é de fundamental importância para que o aumento da síntese proteica seja alcançado. Entretanto, existem divergências na literatura acerca do acréscimo de lisina acima do

requerimento basal do amino ácido, em decorrência do uso da RAC. Ademais, segundo alguns autores o uso da RAC nos últimos 28 dias da fase de terminação dos suínos leva ao processo de dessensibilização dos receptores β-adrenérgicos aos quais a RAC se liga (MOODY; HANCOCK; ANDERSON, 2000; SPURLOCK et al., 1994), portanto, é importante estudar os meios conhecidos para que se busque atenuar os efeitos deste fenômeno.

Com relação ao mecanismo de ação desencadeado após a ligação da molécula de RAC ao receptor celular, as vias de sinalização celular envolvidas ainda não foram estabelecidas totalmente. A completa compreensão do mecanismo de ação celular da RAC poderia ajudar os pesquisadores a entenderem melhor os efeitos observados no suíno vivo.

As hipóteses levantadas são: 1) que a suplementação de lisina em um nível inferior aos preconizados anteriormente pode ser suficiente para que se obtenha resultados ótimos com uso da RAC em programas *step up*; ou seja, com doses crescentes de RAC ao longo do tempo, e 2) a RAC ativa a via de sinalização celular da proteína mTOR nas células musculares estimulando a síntese proteica. Assim, objetivou-se com este trabalho determinar se o uso de programas de suplementação da RAC em esquema *step up,* associado a dois níveis de acréscimo de lisina em relação ao requerimento basal do amino ácido, interferem no desempenho, características de carcaça, parâmetros bioquímicos do sangue e na qualidade da carne, bem como verificar o mecanismo de ação celular da RAC no tecido adiposo subcutâneo e no tecido muscular do músculo *Longissimus dorsi* de suínos em fase de terminação que receberam RAC na dieta por 28 dias.

2 REFERENCIAL TEÓRICO

2.1 Agonistas β-adrenérgicos (ABA)

Os ABA sintéticos são compostos com estrutura química (Figura 1) semelhante à das catecolaminas (SALEM et al., 2006), e possuem a habilidade de se ligarem aos receptores β-adrenérgicos, os quais são receptores de superfície celular acoplados à proteína G estimulatória, havendo três subtipos: β1, β2 e β3 (MILLS et al., 2003). Estas substâncias influenciam especialmente as células musculares e adiposas, apesar de os receptores β-adrenérgicos estarem espalhados pela maioria dos tecidos dos mamíferos, variando a distribuição dos subtipos e suas proporções de acordo com a espécie (MERSMANN, 1998).

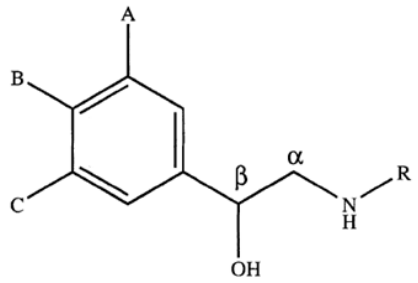

Figura 1 Estrutura geral de um agonista β-adrenérgico

Fonte: (Adaptado de Smith, 1998).
Nota: A, B e C são pontos de substituições no anel aromatic por íons H^+ ou OH^-

Os ABA são utilizados em terapêutica humana como broncodilatadores e como tocolíticos, porém, há mais de duas décadas vem-se estudando os efeitos destas substâncias como aditivo alimentar para animais de produção, com destaque para o salbutamol, o clembuterol e , principalmente a RAC (DUNSHEA et al., 1993), a qual foi aprovada para uso na alimentação de suínos nos E.U.A. em 1999 (SALEM et al., 2006). Os ABA se mostram potentes

indutores do crescimento, sendo nos animais de produção de carne, sobretudo nos suínos, utilizados pelo aumento da eficiência alimentar e rápido incremento de massa proteica corporal em detrimento da deposição de gordura na carcaça (CARR et al., 2009; SEE et al., 2004). Em termos moleculares os ABA usados na alimentação animal atuam em vias metabólicas específicas, principalmente no metabolismo das proteínas, lipídeos e carboidratos, redirecionando os nutrientes da dieta para vias metabólicas que favorecem a síntese [proteica em detrimento da deposição de gordura na carcaça (CARR et al., 2009; GUNAWAN et al., 2007; WATKINS et al., 1990).

2.2 Características da ractopamina

A RAC (Figura 2) é um ABA sintético com comprovada eficiência na produção de carne, proporcionando menor deposição de tecido adiposo na carcaça e maior porcentagem de carne magra (BOHRER et al., 2013).

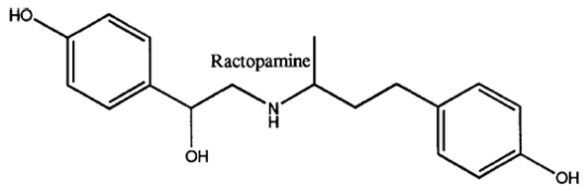

Figura 2 Estrutura química da ractopamina
Fonte: (Adaptado de Smith, 1998).

2.2.1 Características farmacológicas da ractopamina

A absorção da RAC ocorre no intestino delgado dos suínos, uma vez que a molécula ionizada, que é uma base fraca, tem sua ionização reduzida pela

alcalinidade do meio (PALERMO NETO, 2002), apresentando pico plasmático entre uma a três horas após a ingestão da substância (SMITH, 1998).

No fígado a RAC é biotransformada por processo de conjugação hepática com o ácido glicurônico (SMITH, 1998). Dalidowicz et al. (1992) administraram RAC marcada com carbono 14 a suínos e observaram que 88% da dose oral foi eliminada pela urina, ao passo que, por meio de cromatografia líquida de alta performance (HPLC), Smith e Shelver (2002) observaram que nos ovinos e bovinos a eliminação da RAC também parece ocorrer principalmente por via urinária, pois foram encontradas maiores concentrações desta substância nos rins do que no fígado em animais destas espécies, e ainda pode-se detectar a RAC na urina por cinco a sete dias após cessar o fornecimento aos animais (SMITH; SHELVER, 2002).

A ractopamina tem sido considerada uma substância segura nas doses recomendadas considerando os limites máximos de resíduos (LMR) admitidos em tecidos comestíveis. Cerca de uma hora após a interrupção do fornecimento não se encontram mais concentrações da substância ativa, em tecidos comestíveis, capazes de provocar efeitos farmacologicamente importantes em uma pessoa de 60 kg (PALERMO NETO, 2002).

2.2.2 Mecanismo de ação da RAC

Nos suínos a RAC parece se ligar principalmente aos receptores dos subtipos β1 e β2 (SILLENCE, 2004), entretanto apresentam aparentemente efeitos mais consistentes quando ligados aos receptores β2, já que Mills et al. (2003) utilizando células cultivadas, observaram que o aumento da formação do segundo mensageiro cAMP é mais eficiente quando da ligação da RAC ao receptor β2 em relação ao receptor β1.

O mecanismo de ação da RAC postulado (Figura 3) na literatura consiste na estimulação da ação catalítica da enzima adenilato ciclase pela subunidade α da proteína Gs gerando aumento consequente da concentração do monofosfato cíclico de adenosina (cAMP), um potente segundo mensageiro em vias de sinalização celular (MERSMANN et al., 1997; MOODY et al., 2000). O cAMP estimula a liberação da subunidade catalítica da proteína kinase A (PKA) que desencadeia a fosforilação de enzimas intracelulares (LIU et al., 1994; MERSMANN, 1998; MERSMANN et al., 1997; MOODY et al., 2000).

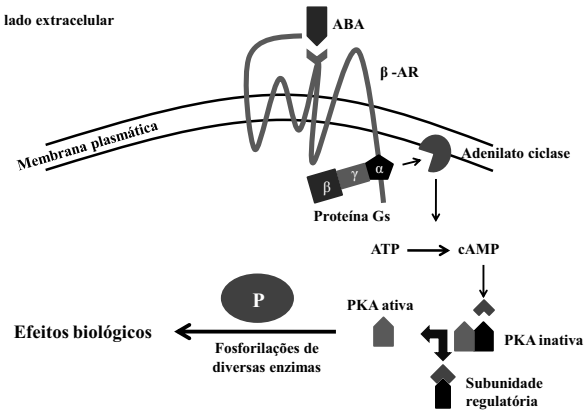

Figura 3 Mecanismo geral de ação dos agonistas β-adrenérgicos, onde: ABA: agonista β-adrenérgico; β-AR: receptor β-adrenérgico; ATP: trifosfato de adenosina; cAMP: monofosfato cíclico de adenosina; PKA: proteína kinase A

Fonte: (Adaptado de Moody et al., 2000).

Entretanto, mais recentemente, muitos indícios apontam para a direção de que a RAC pode exercer um papel importante na ativação de outra via de sinalização celular. Reiter et al. (2007) verificaram que os animais alimentados com ractopamina apresentaram diminuição do mRNA para o transportador de glicose dependente de insulina GLUT-4 no tecido adiposo, que é controlado por uma via metabólica independente da ativação de receptores β-adrenérgicos

estimulados pelos ABA, ou pelo menos, da via de sinalização celular descrita anteriormente. Uma das via metabólicas responsáveis pela ativação do GLUT-4 nos adipócitos e células musculares é acionada após a ligação da insulina ao seu receptor, os quais culminam com a ativação da fosfatidilinositol trifosfato quinase (PI3K) que, por sua vez, leva a ativação de uma cascata de reações em que o alvo é a proteína Akt, também conhecida como proteína quinase B (BRYANT et al., 2002; RICCIARD et al., 2011). A Akt ativada fosforila proteínas da membrana das vesículas citoplasmáticas que contém o GLUT-4, promovendo sua translocação até a membrana citoplasmática (HAJDUCH et al., 2001). Pesquisas prévias que avaliaram a concentração do mRNA do GLUT-4 em suínos que receberam RAC demonstraram tanto a diminuição da expressão do mRNA (REITER et al., 2007) como nenhuma alteração (LIU et al., 1994).

Outra proteína influenciada pela ativação da Akt é a *molecular target of rapamycin*, ou simplesmente mTOR nos mamíferos. A mTOR forma um complexo proteico com outras proteínas e sua via metabólica regula importantes eventos na fisiologia celular envolvidos na síntese proteica, entre eles, os fatores de iniciação e alongamento, e a biossíntese ribossomal (BENTZINGER, 2009; WANG; PROUD, 2006; YANG et al., 2008), em resposta a estímulos hormonais e nutricionais (YANG et al., 2008), sendo comprometida na falta de energia ou nutrientes (WANG; PROUD, 2006). A Akt fosforila uma dessas proteínas, chamada TSC2 inativando-a. A TSC2, juntamente com a TSC1, atua como uma GTPase para a proteína Rheb. Assim, a TSC2 inativada não exerce sua função de GTPase permitindo com que a Rheb mantenha sua função, ou seja, a via da mTOR permanece ativada.

Sabe-se que a via da mTOR é ativada por estimulação da insulina e por aminoácidos (TREMBLAY; MARETTE, 2001). Tremblay et al. (2005) mostraram que a mTOR tem um papel central na síntese proteica, que envolve a ligação de um agonista em um receptor que, por sua vez, dispara a sinalização

em cascata da via de PI3K/Akt. Entretanto, outros pesquisadores demonstraram a ativação da via da mTOR via PI3K em resposta à estimulação do receptor β-adrenérgico (Figura 4), a qual leva à posterior fosforilação e ativação da proteína $P70^{S6K}$ bem como da 4E-BP1 (PAVOINE; DEFER, 2005). A 4E-BP1 quando fosforilada se dissocia do fator de iniciação de transcrição 1, permitindo que este se ligue ao DNA e assim a transcrição é iniciada (CHOO et al., 2008).

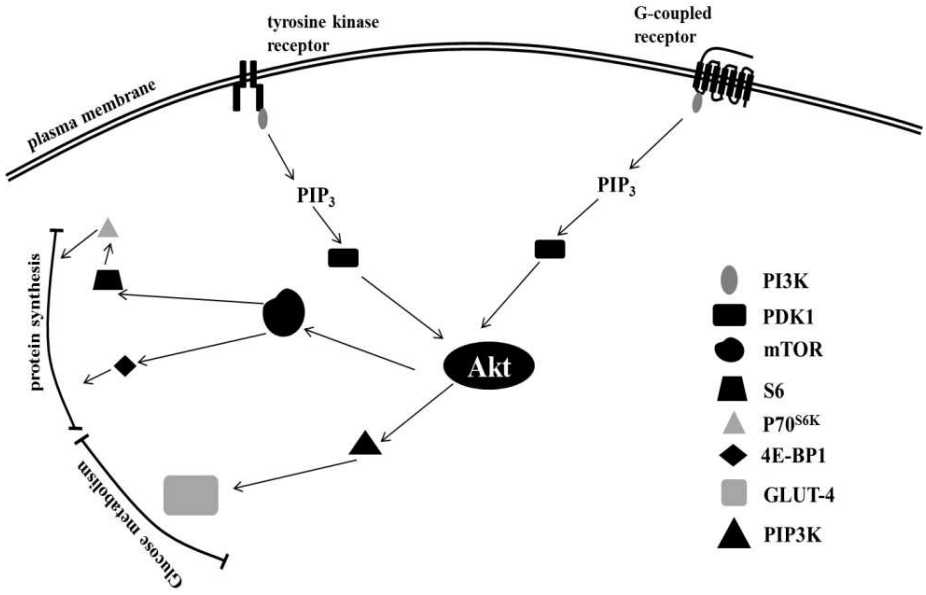

Figura 4 Vias de sinalização celular disparadas pelos receptores tirosina quinase e acoplados à proteína G.

Fonte: A figura foi elaborada a partir das informações contidas em Pavoine e Defer (2005), Tremblay et al. (2005).(Adaptado de cell signalling). [1]

[1] PI3 Kinase Akt signalling. http://www.cellsignal.com/reference/pathway/pdfs/Akt_PKB.pdf (Acessado em 16 de janeiro de 2014).

2.3 Efeito geral da administração da RAC para suínos em terminação

A taxa de síntese de tecido magro em suínos na fase de crescimento é curvilínea, sendo baixa nos pesos mais leves aumentando até um platô máximo, para em seguida declinar rapidamente (VASCONCELOS et al., 2007).

No período final da terminação, a maioria dos animais está na fase estacionária, ou tendendo a fase de declínio para a deposição de carne magra ao passo que, a taxa de deposição da gordura está ascendente (VASONCELLOS et al., 2007)

Segundo Vasconcellos et al. (2007), os ABA podem ser chamados de repartidores de nutrientes, em razão de sua capacidade de redistribuir os nutrientes em função da alteração do metabolismo celular, segundo estes autores há um redirecionamento dos nutrientes que seriam destinados à síntese e deposição de lipídeos para a síntese de tecido muscular. Por este motivo, a RAC é utilizada em suínos em fase de terminação, ou seja, animais que já estão na fase de maior deposição de gordura na carcaça (VASCONCELLOS et al., 2007). De acordo com Vasconcelos et al. (2007) os repartidores de nutrientes tem uma participação estratégica nas formulações das dietas nesta fase porque permitem estender o período de maior taxa de acréscimo de tecido muscular em contrapartida ao tecido adiposo, aumentando o percentual de carne magra na carcaça (HINSON et al., 2012; KUTZLER et al., 2011; ZAGURY, 2002).

O desempenho de animais alimentados com RAC é positivamente afetado, com destaque para o ganho de peso e a eficiência alimentar (ALMEIDA et al., 2013; HINSON et al., 2012). Além disso, suínos alimentados com RAC apresentam carcaças mais magras e mais pesadas (ANDRETTA et al., 2012).

2.3.1 Dessenssibilização dos receptores β-adrenérgicos e uso de protocolos de suplementação em esquema de doses crescentes (*step up*)

Os efeitos da RAC não são constantes durante o período de uso na ração de suínos em terminação. A resposta dos animais inicia-se rapidamente seguida de platô e aparente decréscimo desta resposta (ARMSTRONG et al., 2005). Esse decréscimo se dá provavelmente devido ao processo de dessenssibilização dos receptores ou *down regulation*, ou ambos (MOODY et al., 2000; SPURLOCK et al., 1994).

A suplementação da RAC em programas com doses crescentes do aditivo, chamados de programas *step up*, podem ser utilizados visando mitigar os efeitos de dessenssibilização (ARMSTRONG et al., 2005; POLETTO et al., 2009; SEE et al., 2004). De fato os trabalhos mostram resultados interessantes do ponto de vista da melhora de desempenho e características de carcaça. See et al. (2004) observaram melhoras nestes parâmetros utilizando sistema *step up* de 5 ppm para 10 ppm em relação a um tratamento constante com 11,6 ppm de RAC. Já outros autores testaram programas *step up* também de 5 ppm para 10 ppm, mas o nível constante escolhido foi 5 ppm, observando melhorias tanto no desempenho como nas características de carcaça também (ARMOSTRONG et al., 2005). Esses mesmos autores elaboraram dois sistemas *step up*, 5 ppm por 14 dias seguido de 10 ppm por 21 dias e 5 ppm por 21 dias seguidos de 10 ppm por 14 dias, e não observaram diferença entre estes programas, sendo mais vantajoso o uso da segunda opção por ser o custo menor, uma vez que se usa menor quantidade da RAC.

2.3.2 Efeito da administração de RAC no tecido adiposo de suínos em terminação

No tecido adiposo a RAC é um agente que induz a diminuição da deposição de gordura na carcaça dos animais submetidos à suplementação com a substância (MIMBS et al., 2005; SEE et al., 2004; WEBER et al., 2006).

A intensidade do efeito dos ABA's sobre as células adiposas dos animais está relacionada, entre outros fatores, ao tipo de receptor β-adrenérgico expresso por aquela célula. McNell e Mersmann (1999) utilizando a reação em cadeia da polimerase em tempo real (rtPCR) quantificaram a produção de mRNA para os receptores β-adrenérgicos nos adipócitos de suínos e verificaram a presença de apenas 7% do total de transcritos sendo para receptores do tipo β_3, 73% tipo β_1 e 20% β_2. Para efeito de comparação a quantificação dos receptores β-adrenérgicos, o tecido adiposo de ratos apresenta proporção de mRNA para receptores β_3 32% superior que a de β_1, o que pode explicar o fato da resposta celular do tecido adiposo de ratos aos ABA ser mais acentuada do que nos suínos (LIU et al., 1989).

Liu, Boyer e Mills (1989) sugeriram que os receptores β-adrenérgicos promovem maior efetividade em induzir a lipólise por meio da estimulação da atividade da enzima lipase hormônio sensível, já, segundo outros autores, o efeito predominante da ractopamina sobre o tecido adiposo é a inibição da lipogênese devido à inibição da enzima acetil-CoA carboxilase (MILLS et al., 1990; PETERLA; SCANES, 1990).

Ferreira et al. (2013) realizaram uma revisão sistemática acerca da temática que envolve ser lipogênese ou lipólise responsável pela diminuição da quantidade de tecido adiposo na carcaça de suínos alimentados com dietas contendo RAC. De acordo com os autores, a diminuição se dá mais provavelmente por inibição da lipogênese, uma vez que trabalhos prévios

haviam mostrado a diminuição da concentração de enzimas lipogênicas, como a ácido graxo sintetase e o fator de transcrição de enzimas lipogênicas SREBP-1 (HALSEY et al., 2011; REITER et al. 2007).

Outros autores suspeitaram que a RAC pudesse estimular mais fortemente lipólise, então, após quantificação de ácidos graxos livres (AGL) não observaram aumento destes elementos (DUNSHEA; KING, 1994; 1995), indicando que a lipólise pode não ser o fator preponderante na diminuição da quantidade de tecido adiposo depositado nas carcaças em suínos (FERREIRA et al., 2013).

Já Page et al. (2004) observaram que há aumento de ocorrência da apoptose de adipócitos em camundongos com dieta suplementada com 800 ppm de ractopamina, dose esta bem mais elevada do que a utilizada em suínos. Então, a maior quantidade de receptores β_3, bem como a dose elevada, poderiam ser possíveis causas para o resultado observado (FERREIRA et al., 2013). Por fim, Dunshea e King (1995) sugeriram que a ractopamina não estimula a mobilização de gordura ou lipólise em suínos, ou caso isto ocorra, este efeito desaparece até o terceiro dia de suplementação com RAC.

2.3.3 Efeito da administração de RAC no tecido muscular de suínos em terminação

Pode ser observado na carcaça de suínos suplementados com RAC o incremento no peso da mesma (FERNANDEZ-DUEÑAS et al., 2008) e na porcentagem de carne magra na carcaça (CANTARELLI et al., 2009; MITCHELL, 2009), bem como na área de olho de lombo (HINSON et al., 2011; WEBER et al., 2006), profundidade de lombo (TAVÁREZ et al., 2012) e no rendimento de carcaça (PATIENCE et al., 2009).

Watkins et al. (1990) afirmaram que a ractopamina estimula o aumento da concentração do mRNA da actina e da miosina, levando ao aumento da síntese proteica muscular. Adeola, Ball e Young (1992), estudaram o fluxo da fenilalanina para quantificar a síntese proteica nos músculos bíceps femoral e *longissimus* e verificaram aumento da síntese das proteínas miofibrilares, corroborando com os resultados de Grant et al. (1993) que observaram o aumento da expressão gênica destas proteínas utilizando a técnica de *dot-blot hibridization.*

Gunawan et al. (2007) utilizando rt-PCR em amostras dos músculos *longissimus dorsi* e *semitendinosus* de suínos alimentados com ractopamina, observaram aumento da síntese da cadeia pesada da miosina tipo IIB após 12 horas da ingestão da ractopamina e perdurando por quatro semanas. Estes mesmo autores observaram que a concentração de transcritos de mRNA para a proteína G_S aumentou significativamente em relação ao tratamento controle após 12 horas, voltando ao nível normal após duas semanas e diminuindo significativamente em relação ao tratamento controle após quatro semanas (GUNAWAN et al., 2007).

Adicionalmente, o uso da RAC acarreta em maior retenção de N na carcaça diminuindo sua excreção no ambiente (ROSS et al., 2011) e levando a uma melhor eficiência de utilização deste elemento (CANTARELLI et al., 2009).

2.3.4 Qualidade da carne de suínos em terminação alimentados com dietas suplementadas com RAC

O uso da RAC tem como principal objetivo a melhora do desempenho e das características de carcaça dos animais, porém as características da carne devem ser preservadas para atender os anseios dos consumidores. Os trabalhos

apontam que a RAC não leva a alterações nas características sensoriais da carne suína (APPLE et al., 2008; MADEIRA et al., 2013; WEBER et al., 2006), mesmo que pequenas alterações tenham sido encontradas. Estas alterações se referem a um aumento de 1,1% nos valores de pH na carne suína proveniente de animais suplementados com RAC, bem como alterações na cor quando o método indireto foi utilizado (CARR et al., 2005; WEBER et al., 2006) bem como quando o método direto foi adotado (KUTZLER et al., 2011).

O perfil lipídico da carne de suínos que receberam RAC também parece não ser afetado pelo aditivo (APPLE et al., 2008; WEBER et al., 2006). Todavia Apple et al. (2008) observaram efeito positivo da RAC nos níveis de ácidos graxos poli-insaturados (PUFA). Weber et al. (2006), por sua vez, não obtiveram maior índice de PUFA no toucinho de barriga de suínos alimentados com ractopamina.

Já o marmoreio da carne parece ser uma variável mais subjetiva levando a resultados inconsistentes. No estudo de Apple et al. (2008) os animais alimentados com ractopmina apresentaram maiores índices de marmoreio, o que não foi observado no trabalho de Armstrong et al. (2004), que não observaram efeito da ractopamina nesta variável.

Scramlin et al. (2008) analisaram o efeito da ractopamina na qualidade do bacon se suínos. O rendimento de bacon não foi afetado pelo uso de nenhuma das duas doses (5 e 7,4 ppm) de ractopamina ($P < 0,05$).

2.4 Suplementação de lisina na ração para suínos em terminação alimentados com dietas contendo RAC

A seleção genética para o desenvolvimento de linhagens que resultem em carcaças com maior percentual de carne magra levou também a um incremento no requerimento de aminoácidos essenciais (FERREIRA;

SCHINCKEL, 2013), especialmente a relação lisina / ingestão calórica (SCHINCKEL et al., 2008). Somado a isso, o estímulo da síntese proteica, desencadeado pelo uso da RAC requer um ajuste nos níveis destes aminoácidos, principalmente a lisina (DUNSHEA et al., 1993).

Mitchell et al. (1990) estimaram que um acréscimo de 30% nos níveis de lisina acima dos níveis basais era adequado para suínos em terminação suplementados com RAC. Porém, devido principalmente aos avanços genéticos ao longo dos anos, os requerimentos dos aminoácidos foram revistos. Foi estimado ao redor de 18% o acréscimo de lisina para suínos suplementados com RAC (ROSTAGNO et al., 2011; NRC, 2012).

Equações de predição do acréscimo de proteínas e deposição de gorduras demonstraram que a RAC leva a uma alteração na curva de crescimento de tecido magro, demonstrado por uma marcante alteração na composição centesimal das carcaças dos suínos com aumento da porcentagem de tecido magro em relação à gordura (SCHINCKEL et al., 2003).

3 CONSIDERAÇÕES GERAIS

A RAC é um aditivo alimentar considerado seguro para os suínos, bem como para os consumidores da carne, com efeitos marcantes no desempenho e nas características de carcaça de suínos. Da mesma forma, as características da carne, tanto quanto a qualidade e aceitabilidade, permanecem inalteradas com o uso do produto.

Porém, devido à possibilidade de uso da RAC em diferentes esquemas de suplementação, os requerimentos nutricionais de aminoácidos devem ser atualizados frequentemente. Além disso, um completo conhecimento dos mecanismos pelos quais a RAC exerce seus efeitos representaria um grande avanço científico, já que não estão totalmente elucidados na literatura. Ademais, o conhecimento da via metabólica na atuação de uma substância química pode ser extremamente importante para que outros pesquisadores possam propor novas estratégias de utilização do produto, bem como auxiliar no momento de formular rações.

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SEGUNDA PARTE - ARTIGOS

ARTIGO 1 Ractopamine in step up programs with two levels of additional lysine above basal requirements for finishing barrows: growth performance, carcass traits and molecular stand points

Normas do Journal of Animal Sciences (versão submetida, sujeita a modificações)

The authors want to acknowledge the Brazilian National Council for Scientific and Technological Development (CNPq), to the CAPES Foundation, to the Research Support Foundation of Minas Gerais States (FAPEMIG) for financial support of this research and to the National Institute of Science and Technology (INCT – Brazil). The first author thanks CAPES Foundation for the scholarship conceived for a sandwich exchange program at Purdue University – USA, scholar process 8944-11-3.

ABSTRACT: A 28-day study was conducted to evaluate the effects of three step up levels of ractopamine (RAC) associated to two additional levels of Lys above the basal requirements on growth performance, carcass characteristics and the mechanism of action on adipose and muscle tissue. One hundred-eight finishing pigs (initially 75.37 kg ± 2.88) were used for growth data meanwhile 54 pigs were used for carcass data. Samples from 18 pigs were used for the molecular study. Pigs were blocked by initial BW and allotted to one of the nine treatments: negative control without addition of RAC nor Lys (NC), constant 7.5 ppm RAC, 5 ppm RAC for 14 days followed by 10 ppm for 14 days (step up 1), 5 ppm RAC for 21 days followed by 10 ppm for 7 days (step up 2) and 5 ppm RAC for 7 days followed by 10 ppm for 21 days (step up 3). On constant and step up treatments were added 15 or 30% Lys above basal resulting on a 4x2+1 factorial with six replicates. Loin muscle and fat tissue were collected for carcass characteristics analysis and western blotting for p-AKT, p-P70^{S6K} and CPT-1. RAC fed animals had better GF and energy efficiency of utilization (EF) in the first half of the trial ($P < 0.05$). On the second half greater ADG was observed for RAC-treated animals ($P < 0.05$) except the step up 2 with 30% additional Lys. For the second half of the trial RAC positively affected ADG, GF and EF ($P < 0.01$), while a step 1 vs step 2 effect was observed for ADG ($P < 0.03$). For the overall period RAC-treated pigs had greater ADG compared to NC ($P < 0.05$). An average of 23% improvement on feed efficiency and 30% improvement on energy efficiency of utilization were observed for RAC-fed pigs in comparison to NC ($P < 0.05$). Chilled carcass weight and loin eye area were increased for RAC-fed animals ($P < 0.01$). Western blots

showed a positive effect of RAC on the activation of the mTOR pathway towards stimulation of protein synthesis. A greater amount of the p-P70S6K was found in muscle samples of RAC-fed animals with 15% additional Lys compared to NC ($P < 0.10$). RAC was effective on improving efficiency of production. Lys supplementation of 15% was enough for optimal performance of the pigs in this study. However, step up programs did not outperform RAC constant programs. Results in this study suggest that RAC stimulation of protein synthesis occurs through mTOR signaling pathway.

Key words: pigs, β-agonist, lysine, protein synthesis, mTOR

INTRODUCTION

For decades investigators have been studying the benefits of ractopamine (RAC), a β-adrenergic agonist (BAA), on diets for finishing pigs. The main goal for producers is increasing efficiency (Carr et al., 2009). RAC effects occur secondarily to the activation of β-adrenergic receptors (BAR) by RAC, which lead to a series of specific metabolic events that culminate with decreased fat deposition (Dunshea et al., 1993; Mimbs et al., 2005) and increased lean growth through notably higher myofibrillar protein synthesis (Gunawan et al., 2007). In order to obtain those effects on protein synthesis Lys inclusion levels for RAC-supplemented animals have to be augmented (Mitchel et al., 1990).

In the past researchers verified that RAC activated adenylyl cyclase pathway (Mersmann et al., 1997). However, most recent works have indicated also that BAA, and possibly RAC, is able to activate cell signals through binding to another type of G-protein coupled receptor that activates PI3 kinase pathway. For instance, Miniaci et al. (2013) has shown that CL316,243, a β3-adrenergic agonist, activates protein synthesis through mTOR signaling pathway. Moreover, RAC have been tested in step up doses programs attempting to mitigate down-regulation effects (See et al., 2004; Poletto et al., 2009), which might attenuate RAC response (Spurlock et al., 1994). The hypothesis highlighted in this work is that RAC also activates the mTOR pathway and that a lower level of additional Lys combined to step up programs might have similar results than those found in the literature.

The aim of this work was to verify if step up doses of RAC associated to 2 additional levels of Lys above the basal requirements interfere on growth performance and carcass traits of finishing pigs, as well as to verify whether RAC is able to activate the mTOR signaling pathway on adipose and muscle tissue.

MATERIAL AND METHODS

All procedures and housing adopted in this experiment were approved by the "Ethic Committee on Animal Use" of Federal University of Lavras, Lavras – Brazil.

Animals and housing

The experiment was carried out between November and December of 2011 at the Swine Experimental Center of the Animal Science Department of Federal University of Lavras, in Lavras – Brazil. One hundred and eight barrows from a high lean genetic line weighing 75.37 kg ± 2.88 were divided in blocks according their initial weight. Two pigs were housed in each one of the pens with 1m x 3m of dimension and 1m height, with water nipples and semi-automatic feeders. All animals were considered for growing data, but 54 animals (1 pig per pen) were used for carcass data, representing all of the experimental units.

Treatments

Treatments were defined in order to have three different RAC step up feeding programs and two levels of additional Lys above basal levels for each program. Step up 1 (S1) consisted of 5 ppm RAC for 14 days followed by 10 ppm RAC for 14 days, with 15 and 30% Lys (S1-15 and S1-30 respectively). Step up 2 consisted of 5 ppm RAC for 21 days followed by 10 ppm RAC for 7 days, with 15 and 30% Lys (S2-15 and S2-30 respectively). Step up 3 consisted of 5 ppm RAC for 7 days followed by 10 ppm RAC for 21 days, with 15 and 30% Lys (S3-15 and S3-30 respectively). Moreover, a positive control with constant level of 7.5 ppm RAC with 15% or 30% Lys above basal levels (PC15 and PC30 respectively) and a negative control with no RAC neither additional Lys accretion (NC) were used.

Experimental design

The trial was designed on randomized blocks in a 4x2+1 augmented factorial arrangement consisting of four RAC programs and two levels of Lys supplementation above the basal level. The augmented factorial was represented by the addition of the negative control treatment. There were 9 treatments and 6 replicates for each treatment. The experimental unit was the pen.

Diets

The negative control diet was formulated with a lower metabolizable energy (ME) level compared to RAC diets, which were formulated to be isoproteic and isoenergetic, to meet or exceed nutrition requirements according Rostagno et al. (2011). Methionine, threonine and tryptophan were added according to Lys levels. Treatments in which Lys were added in 30% above basal levels needed additional extra amount of tryptophan as well (Table 1).

Procedures

The experimental period lasted for 28 days, in which the animals had ad libitum access to feed and water. At d13, pigs were fasted for 12 hours and weighed. At the end of experimental period 54 animals were fasted for 12 hours, weighed, and then shipped to a commercial facility to be slaughtered according to the Brazilian legislation (BRASIL, 2000). Weight data and feed waste were assessed in order to evaluate growth performance criteria, which were final weight (BW), average daily gain (ADG), average daily feed intake (ADFI), gain to feed ratio (G:F), Lys efficiency of utilization (LEU) and energy efficiency (EF = energy ingestion (Mcal) / ADG), for the first 14 days of experiment, for the second 14 days, as well as the overall of the entire experimental period.

Table 1. Experimental diets for finishing pigs fed ractopmine (RAC) on step up programs and two additional levels of lysine above basal total lysine requirements.

Ingredients (%)	Basal	15% lysine above basal			30% lysine above basal		
		5 ppm	7,5 ppm	10 ppm	5 ppm	7,5 ppm	10 ppm
Corn	82.000	75.500	75.500	75.500	75.500	75.500	75.500
Soybean, 46%	14.150	19.600	19.600	19.600	19.600	19.600	19.600
Soybean oil	0.570	1.900	1.900	1.900	1.900	1.900	1.900
Phosphate	0.728	0.835	0.835	0.835	0.835	0.835	0.835
Calcium	0.590	0.620	0.620	0.620	0.620	0.620	0.620
Sodium chloride	0.329	0.330	0.330	0.330	0.330	0.330	0.330
Mineral and vitamin premix	0.500	0.500	0.500	0.500	0.500	0.500	0.500
DL-Methionine 99	0.018	0.033	0.033	0.033	0.068	0.068	0.068
L-Lysine 78	0.278	0.255	0.255	0.255	0.397	0.397	0.397
L-Threonine 98	0.058	0.063	0.063	0.063	0.139	0.139	0.139
Tryptophan 98	0.000	0.000	0.000	0.000	0.0155	0.0155	0.0155
Tylan G250®*	0.040	0.040	0.040	0.040	0.040	0.040	0.040
Clay	0.739	0.299	0.274	0.249	0.030	0.005	0.000
Ractosuin®**	0.000	0.025	0.050	0.075	0.025	0.050	0.075
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Metabolizable energy, Kcal/Kg	3,230	3,300	3,300	3,300	3,300	3,300	3,300
Crude protein, %	13.00	15.00	15.00	15.00	15.00	15.00	15.00
Lysine, %	0.737	0.848	0.848	0.848	0.958	0.958	0.958
Methionine, %	0.228	0.263	0.263	0.263	0.297	0.297	0.297
Threonine, %	0.494	0.568	0.568	0.568	0.642	0.642	0.642
Tryptophan, %	0.130	0.153	0.153	0.153	0.172	0.172	0.172
Available phosphorus, %	0.226	0.250	0.250	0.250	0.250	0.250	0.250
Calcium, %	0.463	0.512	0.512	0.512	0.512	0.512	0.512

* Tilosin 250 g/Kg of product, Elanco Brasil, São Paulo, Brazil.
** Ractopamine 2%, Ourofino Saúde Animal, Cravinhos, Brazil.

After the experimental period the animals were transported for 8 km to the slaughter. Pigs were killed after 12 hours of fasting. Whole

carcasses were kept into the chilling room at 0-1°C. Chilled carcasses were weighted (CCW) after been kept 24 hours at the chilling room and calculated dressing percentage (DP). Backfat thickness at tenth rib (BFT) was measured as well as loin depth (LD) with an electronic paquimeter. Loin eye area (LEA) was evaluated drawing the outline of the muscle at the tenth rib on a plastic paper and then scanning and measuring the area through ImageJ IJ 1.46r (Rasband and Ferreira, 2012). Approximately 30 g of loin muscle and subcutaneous fat at the tenth rib were harvested, immediately snap frozen in liquid nitrogen and then stored at -80°C, for western blot analysis.

Western blots

Adipose and muscle tissues samples from NC, PC15 and PC30 (n = 18) were homogenized with extraction buffer containing protease and phosphatase inhibitors (PBS + Protease Inhibitor Cocktail #P8340, Sigma-Aldrich, St. Louis, MO + 10mM NaF). Protein concentration was assessed according to Bradford assay using microplates in a BioTek SX2 microplate reader (BioTek U.S., Winooski, VT). Thirty micrograms of protein were diluted in Laemlli buffer (0.125 M Tris HCl pH 6.8, 4% SDS, 20% glycerol, 10% 2-mercaptoethanol and 0.004% bromphenol blue), heated at 98°C for 5 min, loaded and separated into a 8-10 % SDS-polyacrylamide gel electrophoresis using a Mini Trans-Blot® Cell (BioRad, Hercules, CA) at 100V for approximately 1.5 h. Proteins were transferred from the gel to a nitrocellulose membrane (Bio-Rad Laboratories - Hercules, CA, USA) using the same equipment used for

SDS-PAGE at 100 V for 1 h. Membranes were blocked with 5 % defatted powder milk + TBS solution, washed three times for 10 min with TBST buffer (TBS + 1% of Tween 20), and then incubated overnight at 4°C with rabbit IgG primary antibodies diluted 1:1000. Primary polyclonal antibodies used were anti phospho-Akt 1/2/3 (Ser 473), anti phospho-$P70^{S6K}$ (α C-18), anti CPT-1 (H-40), anti α-tubulin (H-300) and anti β-actin. Primary antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA), except for β-actin (Sigma-Aldrich, St Louis. MO). Membranes were washed three times with TBST and then incubated at room temperature for 1 hour with HRP linked anti rabbit secondary antibody (Sigma-Aldrich, St Louis. MO). Proteins were visualized using chemiluminescent HRP substrate (EMD Millipore Corporation, Billerica, MA, USA) after development on a radiographic film (Eastman Kodak, Rochester, NY). The optical density of the bands was determined using ImageJ 1.46r (Rasband and Ferreira, 2012). In order to normalize the results α-tubulin or β-actin primary antibody was used for each membrane analyzed.

Statistical analysis

Data were analyzed by ANOVA using proc Mixed of SAS. Non-significant interactions at 5% were excluded from the model. Any variable that failed to have a normal distribution was analyzed through the non-parametric test of Kruskal-Wallis at 5% of significance in SPSS statistical package. Differences between treatment means were tested by Duncan test considering 5 % of level of significance. Initial weight was

used as a covariate for the second week data analysis. Linear orthogonal contrasts were formulated to analyze different aspects of the data. First, negative control was compared to others in order to determine whether RAC had an effect or not; second contrast was formulated to analyze Lys addition effect (15 vs 30%); third contrast compared the three step up treatments with 15% Lys accretion; forth contrast compared the three step up treatments with 30% Lys accretion; fifth contrast compared all the three step up programs with Lys accretion rates combined agains PC15 and PC30 combined; sixth contrast compared step up 1 vs step up 2; and seventh contrast compared step up 1 vs step up 3. Finally, means for the molecular study were compared through Tukey test ($\alpha = 0.10$).

RESULTS AND DISCUSSION

All evaluation criteria were normally distributed except GF and EF for the overall period (Table 2). There were no Lys x RAC interaction for any of the growth criteria evaluated in this study ($P > 0.05$), and were therefore excluded from the model.

On the first 14 days of the trial GF and the energy efficiency of utilization were improved on all RAC-fed animals ($P < 0.05$), despite it is known that extra caloric effect accounts for better digestibility of ingredients of the diet as well as a higher efficiency on the utilization of the energy (Azain, 2004).

Table 2. Growth performance of finishing pigs fed ractopamine (RAC) on step up programs and two levels of lysine (n=6).

Variable	NC	Additional lysine								SEM	*P* value		
		15%				30%							
		PC	S1	S2	S3	PC	S1	S2	S3		R	L	R*L
Day 0 to day 13													
FW, kg	87.92 C	91.04 AB	90.63 AB	88.92 BC	91.75 A	92.17 A	90.96 AB	90.58 AB	91.00 AB	0.52	**	NS	NS
WG, kg	13.21 B	15.17 AB	15.13 AB	14.67 AB	16.17 A	16.63 A	15.38 AB	15.25 AB	15.04 AB	0.25	*	NS	NS
ADG, kg	0.94 B	1.08 AB	1.08 AB	1.09 AB	1.15 A	1.19 A	1.10 A	1.13 A	1.07 AB	0.02	*	NS	NS
ADFI, kg	2.73	2.62	2.46	2.73	2.66	2.70	2.51	2.61	2.57	0.04	NS	NS	NS
G:F	0.34 B	0.42 A	0.44 A	0.41 A	0.44 A	0.44 A	0.44 A	0.43 A	0.42 A	0.007	**	NS	NS
LEU, g_{lw}/g_l	46.66	49.34	51.88	47.87	51.41	52.36	51.77	51.19	49.38	0.67	NS	NS	NS
EF, Kg/MCal	0.11 B	0.13 A	0.13 A	0.12 A	0.13 A	0.13 A	0.13 A	0.13 A	0.13 A	0.002	**	NS	NS
Day 14 to day 27													
FW, kg	-	-	-	-	-	-	-	-	-	-	-	-	-
WG, kg	14.58 C	18.00 AB	18.38 AB	17.96 AB	19.50 A	17.58 AB	18.88 A	16.25 BC	18.42 AB	0.28	**	NS	NS
ADG, kg	1.04 C	1.29 AB	1.31 AB	1.28 AB	1.39 A	1.26 AB	1.35 A	1.16 BC	1.32 AB	0.02	**	NS	NS
ADFI, kg	3.01 AB	3.13 A	3.14 A	2.97 AB	3.07 AB	2.96 AB	3.11 A	2.72 B	2.98 AB	0.04	*	NS	NS
G:F	0.35 B	0.41 A	0.42 A	0.44 A	0.45 A	0.42 A	0.43 A	0.43 A	0.44 A	0.006	**	NS	NS
LEU, g_{lw}/g_l	47.64	48.68	49.45	51.36	53.32	50.05	50.95	50.45	52.22	0.59	NS	NS	NS
EF, Kg/MCal	0.11 B	0.13 A	0.13 A	0.13 A	0.14 A	0.13 A	0.13 A	0.13 A	0.14 A	0.002	**	NS	NS
Overall													
FW, kg	101.75 B	107.54 A	107.79 A	106.88 A	109.96 A	108.96 A	107.42 A	106.21 A	106.88 A	0.72	**	NS	NS
WG, kg	26.83 C	32.88 AB	32.83 AB	32.63 AB	35.58 A	34.21 AB	33.54 AB	31.13 B	32.79 AB	0.50	**	NS	NS
ADG, kg	0.96 C	1.18 AB	1.17 AB	1.17 AB	1.27 A	1.22 AB	1.20 AB	1.11 B	1.17 AB	0.02	**	NS	NS
ADFI, kg	2.81	2.82	2.79	2.85	2.98	2.83	2.78	2.67	2.74	0.03	NS	NS	NS
G:F	0.34 b	0.42 a	0.42 a	0.41 a	0.43 a	0.43 a	0.43 a	0.42 a	0.43 a	0.005	-	-	-
LEU, g_{lw}/g_l	46.16 B	49.36 AB	49.78 AB	48.26 AB	50.50 A	51.01 A	50.71 A	49.22 AB	50.36 A	0.44	**	NS	NS
EF, Kg/MCal	0.10 b	0.13 a	0.13 a	0.13 a	0.13 a	0.13 a	0.13 a	0.13 a	0.13 a	0.002	-	-	-

* $P < 0.05$; ** $P < 0.01$; NS = non-significant.

Within a row means without a common capital letter differ by Duncan test ($P < 0.05$) when the variable was normally distributed, Kruskal-Wallis ($P < 0.05$) otherwise.

NC = negative control (no RAC or additional lysine added); PC15, PC30 = program constant (7.5 ppm RAC) + 15 or 30% additional lysine; S1-15, S1-30 = step up 1 (5 ppm for 14 days followed by 10 ppm for 14 days) + 15 or 30% additional lysine; S2-15, S2-30 = step up 2 (5 ppm RAC for 21 days followed by 10 ppm RAC for 7 days) + 15 or 30% additional lysine; S3-15, S3-30 = step up 3 (5 ppm RAC for 7 days followed by 10 ppm RAC for 21 days) + 15 or 30% additional lysine.

FW = final weight; WG = weight gain; ADG = average daily gain; ADFI = average daily feed intake; GF = gain to feed ratio; LEU = lysine efficiency of utilization, l = lysine, lw = live weight; EF = energy efficiency of utilization.

Body weight on d 13 were greater for all groups compared to negative control except for S2-15 ($P < 0.05$), meanwhile ADG were greater for two groups in relation to negative control, the PC30 and S3-15 ($P < 0.05$). However, there were no differences ($P > 0.05$) between any of the treatments and the negative control for ADFI and Lys efficiency of utilization. Contrast analysis showed positive effect of RAC ($P < 0.01$) for weight gain, ADG, GF and energy efficiency of utilization (Table 3).

On the second half of the experimental period the WG and the ADG were greater for all treatments compared to NC ($P < 0.05$) except for S2-30. All treatments were superior to the NC for GF and EF ($P < 0.05$). Contrast analysis on the second half of the trial data showed positive effect of RAC, and step up 1 over step up 2 significant effect ($P < 0.01$ and $P < 0.03$, respectively) for WG and ADG, as well as RAC effect for GF and EF ($P < 0.01$), as expected. Considering the overall period it can be noticed that the use of RAC led to heavier animals compared to NC since all treatments had greater BW at d 27 than the NC ($P < 0.05$), however S2-30 group presented a smaller WG compared to S3-15, and NC group presented the smallest WG between all treatments ($P < 0.05$). The same response to WG was seen for ADG, S2-30 was smaller than S3-15 and NC group had the smaller ADG compared to all other treatments ($P < 0.05$).

Table 3. Contrast analysis of growth performance evaluation criteria of finishing pigs fed ractopamine (RAC) on step up programs and two levels of Lys (n=6).

Item	*P* values of contrasts						
	RAC effect	Lys level effect	Lys step up 15 effect	Lys step up 30 effect	Step up effect	Step up 1 vs Step up 2	Step up 1 vs Step up 3
				Day 0 to day 13			
FW	0.09	0.60	0.74	0.48	0.47	0.52	0.72
WG	0.01	0.57	0.86	0.10	0.29	0.69	0.63
ADG	<0.01	0.55	0.65	0.11	0.41	0.71	0.63
ADFI	0.39	0.83	0.98	0.35	0.50	0.16	0.31
G:F	<0.01	0.46	0.65	0.50	0.87	0.26	0.48
LEU	0.07	0.47	0.66	0.51	0.87	0.27	0.49
EF	<0.01	0.46	0.65	0.50	0.87	0.26	0.48
				Day 14 to day 27			
FW	-	-	-	-	-	-	-
WG	<0.01	0.17	0.44	0.74	0.44	0.03	0.63
ADG	<0.01	0.16	0.46	0.76	0.46	0.03	0.63
ADFI	0.98	0.11	0.61	0.87	0.63	0.02	0.38
G:F	<0.01	0.86	0.19	0.57	0.18	0.69	0.15
LEU	0.10	0.86	0.19	0.57	0.19	0.69	0.15
EF	<0.01	0.86	0.19	0.57	0.18	0.69	0.15
				Overall period			
FW	0.01	0.66	0.79	0.39	0.68	0.62	0.71
WG	<0.01	0.53	0.58	0.24	0.66	0.30	0.43
ADG	<0.01	0.50	0.57	0.23	0.65	0.29	0.42
ADFI	0.96	0.13	0.62	0.35	0.75	0.76	0.44
G:F	-	-	-	-	-	-	-
LEU	<0.01	0.35	0.92	0.54	0.72	0.25	0.88
EF	-	-	-	-	-	-	-

FW = final weight; WG = weight gain; ADG = average daily gain; ADFI = average daily feed intake; GF = gain to feed ratio; LEU = lysine efficiency of utilization, Lys = lysine, lw = live weight; EF = energy efficiency of utilization.

Feed efficiency and EF results were similar for both criteria; all treatments had greater GF and EF than the NC ($P < 0.05$). GF values varied from 0.41 to 0.43 for RAC-treated animals and it was 0.34 for the NC, which means an average of 23.53% improvement on feed efficiency. On the other hand, EF values were 0.13 for all RAC-treated animals and 0.10 for the NC, i.e. 30% of improvement in this variable. There were highly significant RAC effects on the contrast analysis for BW, WG, ADG and LEU ($P < 0.01$).

It can be noticed that RAC presented its remarkable effects on growth, especially ADG and GF, corroborating with the most recent works (Hinson et al., 2012; Almeida et al., 2013; Garbossa et al., 2013). Moreover, it is interesting to point out that on the first half of the trial there were no differences on growth performance among the step up groups, neither differences between step up programs vs constant programs nor differences between 15% and 30% Lys groups. However, on the second half of the trial the step up 2 was slightly poorer in terms of performance than step up 1 and Step up 3. See et al. (2004) compared step up, step down and constant programs of RAC supplementation, they found that step up and constant resulted on better performance, which at some point corroborates with the results presented in this paper, even though the authors of this work did not tested step down programs. On the second 14 days in a 28-d step up program, likewise step up 1 in this work, protein accretion increase (Schinckel et al., 2006). The use of 5 ppm of RAC for 21 days followed by 7 days with 10 ppm RAC does not seem to be a good option. This is likely due to the down regulation that occurs following pigs' β-adrenergic continuous ingestion (Spurlock et al., 1994),

once animals fed 10 ppm for the last 7 days did not achieve the same performance than those fed 10 ppm for 14 or 21 days.

On the other hand See et al. (2004) and Canchi et al. (2010) have suggested that RAC use on step up protocols could be a manner of mitigating the effects of desensitization of β-adrenergic receptors. However, we are not able to find differences between the step up programs and constant treatments for growth performance. It was chosen to use the average of the dose from the step up programs, so that the doses of the step up programs were 5 and 10 ppm, and the constant programs doses were 7,5 ppm and the effects might have been balanced.

Looking at the results focusing on Lys supplementation beyond the basal level, it is clear that the use of 30% additional Lys was not required in this experiment. The use of 15% additional Lys was enough, not to mention that it was better sometimes, to attain the optimal growth performance. In fact according to the most recent "Nutrient requirement of swine" (NRC, 2012) the Lys requirement for pigs fed RAC does not go above 18% for high lean selected pigs. Besides, there were no differences on LEU or EF for either the first and second 14 days ($P > 0.05$). However, considering the overall period, pigs that received 30% additional Lys, except for the S2-30, had higher values on LEU compared to the control group ($P < 0.05$), which means that these treatments (PC30, S1-30, S3-30) were less efficient at using Lys.

Contrast analysis showed effect when step up 1 was compared to step up 2 for WG, ADG and ADFI ($P < 0.05$), the lower intake by step up 2 group, despite the authors cannot give an explanation for that, might be the reason for this result in ADG and WG.

Such as for growth data, there were no significant RAC x Lys interaction ($P > 0.05$) for carcass characteristics, been the interactions removed from the model allowing more reliable inferences since additional degrees of freedom were accounted for the error.

It was observed through the contrast analysis for carcass characteristics an extremely significant RAC effect for CCW ($P < 0.0001$) (Table 4). In fact, all treatments had greater ($P < 0.05$) CCW when compared to NC (Figure 1).

Table 4. Contrast analysis of carcass characteristics data of finishing pigs fed ractopamine (RAC) on step up programs and two levels of lysine (n=54).

Item	*P* value of contrasts						
	RAC effect	LYS level effect	Lys step up 15 effect	Lys step up 30 effect	Step up effect	Step up 1 vs Step up 2	Step up 1 vs Step up 3
CCW (kg)	< 0.0001	0.35	0.84	0.48	0.52	0.85	0.23
DP	0.20	0.87	0.11	0.79	0.35	0.07	0.87
BFT (cm)	0.10	0.47	0.52	0.11	0.11	0.80	0.09
LD (cm)	0.03	0.41	0.43	0.30	0.20	0.76	0.50
LEA (cm^2)	<0.01	0.20	0.57	0.37	0.30	0.65	0.51

CCW = chilled carcass weight; DP = dressing percentage; BFT = backfat thickness; LD = loin depth; LEA = loin eye area.

DP was greater for PC15 and S2-30 compared to the NC ($P < 0.05$). Step up 1 had a tendency ($P = 0.07$) for smaller DP than step up 2. Only PC15 had BFT smaller than NC ($P < 0.05$). On the other hand, for LD there was only difference between S2-30 and NC ($P < 0.05$). Finally,

NC and S2-15 had smaller LEA ($P < 0.05$) compared to all other treatments, however, S2-30 was also higher than S1-30.

There were highly significant RAC effects for CCW and LEA ($P < 0.001$), and also RAC effect for LD ($P < 0.05$). NC had the lightest CCW ($P < 0.05$), which was expected due to the RAC effects. NC, S1-30 and S3-15had lower DP than PC15 and S2-30 ($P < 0.05$). S2-30 group mean for LD was higher compared to NC ($P < 0.05$), what seems to agree with the LEA data, which pointed out that S2-30 was greater ($P < 0.05$) than NC, S1-30 and S2-15. For BFT data is very similar between the treatments, that were statistically equal ($P > 0.05$), except for a decrease in BFT for PC30 compared to NC ($P < 0.05$).

Carcass weight and LEA increments corroborates with the meta-analysis conducted by Andretta et al. (2012), even though it was not detected in this study a reduction on the backfat thickness which was indeed observed in other studies (Patience et al., 2009, Andretta et al., 2012). In many cases slight differences demand a large number of animals in a trial to be detected, thus directly influence the inferences made over observations.

Indeed, when added to feed for finishing pigs RAC presents remarkable results on growth performance and carcass characteristics, with outstanding improvements on weight gain and feed efficiency (Kutzler et al., 2010; Hinson et al., 2012), loin eye area (Weber et al., 2006; Hinson et al., 2011), loin depth (Tavárez et al., 2012) and carcass yield (Patience et al., 2009). Additionally, RAC leads to greater retention in the carcass and lower excretion of nitrogen into the environment (Cantarelli et al.2009; Ross et al., 2011). In this study some differences

towards desirable carcass characteristics could be found, especially those comparisons between RAC and NC, however step up programs did not result in improvements in carcass compared to constant programs. On the other hand the use of 15% additional Lys levels above basal levels are as effective as the 30% level for carcass results, which is important for those producers that still use more than 15% Lys above basal levels, because it would diminish expenses and waste of the product, considering that Mitchell et al. (1990) estimated Lys requirements for RAC-fed pigs as 30% higher meanwhile Rostagno et al. (2011) estimated approximately 18% higher than the basal Lys requirements.

Western blots analysis (Figure 2) pointed out particularities of adipose and muscle tissues. In adipose tissue p-Akt was 15% greater on NC samples than PC15 samples ($P < 0.10$), on the other hand, in muscle samples p-Akt was 33% greater on PC15 samples compared to NC samples ($P < 0.10$). PC30 p-Akt was 8% smaller than control on adipose tissue and 12% higher on muscle tissue compared to NC, following a similar pattern p-Akt had on PC15 samples, although they were not statistically different from NC ($P > 0.10$). p-$P70^{S6K}$ was not different among treatments on adipose tissue ($P > 0.10$). It was 15% greater for PC15 than NC on muscles samples though ($P < 0.10$). PC30 had 6% more p-$p70^{S6k}$ than NC, although they were not significantly different ($P > 0.10$).

Reiter et al. (2007) fed RAC to pigs and observed a smaller concentration of the glucose transporter GLUT-4 on adipose tissue as well as on muscle tissue. GLUT-4 is activated through PI3K/Akt signaling pathway, which can be activated secondarily to the stimulation

of the insulin receptor and its substrate IRS-1 (Tremblay et al., 2005) or by G-protein coupled receptors (Gelinas et al., 2007). This pathway has an intermediate effector, the Akt protein or protein kinase B (Ricciardi et al., 2011) which in turn leads to the activation of mTOR pathway. Besides mTOR having influence on glucose metabolism (Tremblay et al., 2005) it has been shown to play a central role on protein synthesis either, what involves an agonist binding to a β_2-adrenergic receptor and the following activation of PI3K/Akt pathway, with subsequent activation of $P70^{S6K}$ and 4E-BP1 (Pavoine and Defer, 2005). In spite of what literature brought decades behind regarding activation of adenylyl cyclase (Mersmann et al., 1997; Moody et al., 2000), results of p-Akt and p-$P70^{S6K}$ taken together clearly show a more intense activation of the mTOR pathway on loin muscle tissue rather than on adipose tissue. In a matter of fact Zhang et al. (2011) have reported the PI3K pathway activation through β_2-adrenergic receptor in mouse myocytes, but not in lungs of liver. Considering that in terms of tissue loin and heart are both muscles, the results here presented are in agreement with Zhang et al. (2011). Therefore, protein synthesis is apparently more effectively stimulated on muscle tissue than on adipose tissue through the mTOR pathway. Figure 3 shows the western blot membranes images for p-Akt and p-$p70^{S6K}$.

A higher protein synthesis, combined or not to a smaller protein turnover rate, is required for muscle growth or hypertrophy. Thus, these results were expected once RAC has been known as a feed additive that remarkably contributes with growth (Almeida et al., 2013) resulting in muscle tissue increments, including efficiency in muscle growth

(Schinckel et al., 2003). In fact the PI3K/Akt pathway, in which $p70^{S6K}$ is a downstream effector, has been shown to be involved on protein synthesis in the liver (Reiter et al., 2004) as well as protein synthesis and fiber hypertrophy on muscle (Miniaci et al, 2013). For CPT-1 there were found no difference between treatments and NC ($P > 0.10$), either for adipose tissue and muscle tissue. CPT-1 and acyl-CoA dehydrogenase (ACDH) are two enzymes that play an important role on lipolysis, regulating the transport of fatty acids from the cytoplasm through the mitochondria membrane so that they are able to be oxidized by β-oxidation. Reiter et al. (2007) did not find differences in CPT-1 on muscle and fat tissues of pigs fed RAC for 52 days, however, they did found differences on ACDH. Reiter et al. (2007) were also able to study and find differences on lipogenic enzymes expression, such as fatty acid synthase (FAS) and sterol regulatory element-binding protein (SREBP-1), so that these lipogenic enzymes were more expressed on RAC-fed pigs than in control pigs. Ferreira et al. (2013) have already pointed out through a systematic review that the effects observed on adiposity of RAC-fed pigs are likely due to a lipolysis inhibition rather than a lipolysis stimulation process.

The compilation of the results found in literature and the work presented herein suggest that the effects of RAC on adipose and muscle tissue might occur through different signaling pathways. According to Mersmann et al. (1997), β-adrenergic agonists bind to β-adrenergic receptors and activate adenylyl cyclase increasing intracellular cAMP, which in turn activates PKA, leading to phosphorylation of several enzymes that inhibits anabolic lipid metabolism. Researches in this lipid

metabolism field and RAC showed decreased expression of lipogenic enzymes in adipose tissue such as FAS (Reiter et al., 2007, Halsey et al., 2011), which has been demonstrated to be inversely related to cAMP levels in the cell, either by inhibition of FAS gene expression at transcriptional level (Foretz et al., 1998) or by phosphorylation, and consequent inactivation, of acetyl-CoA carboxylase (Kim et al., 1989). On the other hand, mTOR pathway, a protein synthesis pathway activated by the PI3K, was shown to be activated on RAC-fed pigs in this study.

Conclusions

Once more RAC has proven to be effective on improving efficiency of pig production. Lys supplementation of 15% was enough for optimal performance of the finishing pigs in this study, as well as the step up programs did not overcome 7.5 ppm RAC constant programs.

Additionally, results in this study suggest that RAC stimulation of protein synthesis occurs through mTOR signaling pathway.

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Figure 1

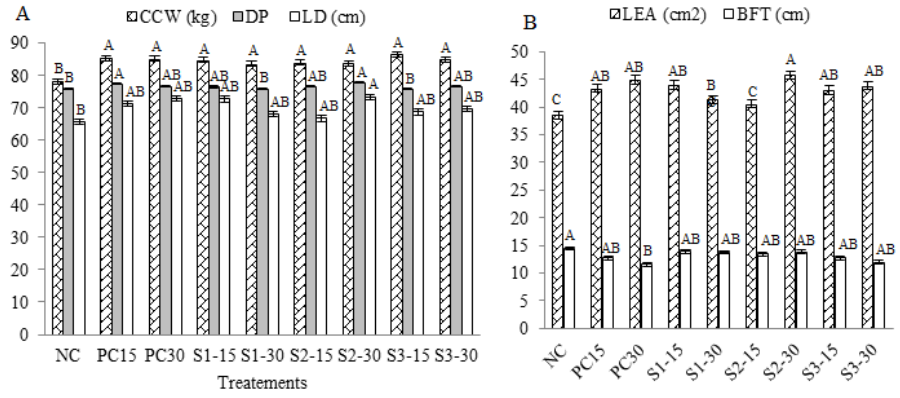

Figure 1. Carcass characteristics of finishing pigs fed ractopamine for 28 days in step up programs and two levels of lysine. A: chilled carcass weight (CCW), dressing percentage (DP), loin depth; B: loin eye area (LEA) and backfat thickness (BFT). Treatments not sharing same capital letter differ by Duncan test ($\alpha = 0.05$). NC = negative control (no RAC or additional lysine added); PC15, PC30 = program constant (7.5 ppm RAC) + 15 or 30% additional lysine; S1-15, S1-30 = step up 1 (5 ppm for 14 days followed by 10 ppm for 14 days) + 15 or 30% additional lysine; S2-15, S2-30 = step up 2 (5 ppm RAC for 21 days followed by 10 ppm RAC for 7 days) + 15 or 30% additional lysine; S3-15, S3-30 = step up 3 (5 ppm RAC for 7 days followed by 10 ppm RAC for 21 days) + 15 or 30% additional lysine.

Figure 2

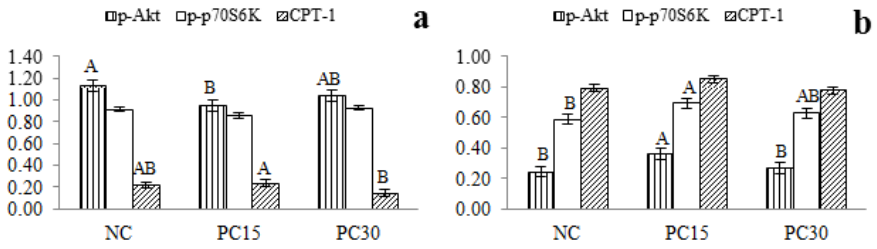

Figure 2. Pixel density arbitrary values for protein expression obtained from western blot analysis of adipose tissue (a), and loin muscle tissue (b) from finishing pigs fed 7.5 ppm of ractopamine (RAC) and two levels of lysine for 27 days. Phospho-Akt (p-Akt), phospho-P70S6K (p-P70S6K) and carnitine palmitoyltransferase I (CPT-1) were evaluated. NC, negative control + basal level of lysine; PC15, 7.5 ppm RAC + 15% additional lysine; PC30, 7.5 ppm RAC + 30% additional lysine. Means followed by different capital letter are different by Tukey α=0.10.

Figure 3

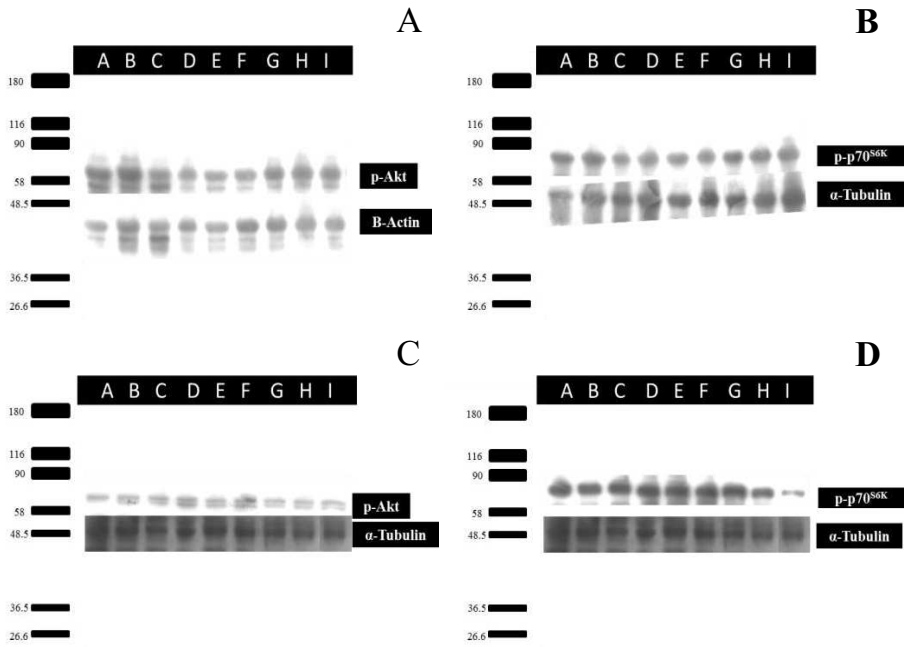

Figure 3. Western blot membranes of adipose tissue p-Akt (A), adipose tissue p-p70^{S6K} (B), loin muscle p-Akt (C) and loin muscle p-p70^{S6K} (D). Endogenous control was made with β-actin for adipose p-Akt and α-tubulin for other proteins. Tissues were collected from finishing pigs fed 7.5 ppm of ractopamine (RAC) and two levels of lysine for 28 days. Lanes A, B and C represent negative control (no RAC or additional lysine); lanes D, E and F represent constant RAC program PC15 (7.5 ppm RAC + 15% additional lysine; G, H and I represent constant RAC program PC30 (7.5 ppm RAC + 30% additional lysine).

Artigo redigido em co-autoria com Thiago dos Santos Araújo, Ana Cláudia Alves, Laura Cristina Porto, Allan Paul Schinckel, Zach J. Rambo, Vinícius de Souza Cantarelli, Márcio Gilberto Zangeronimo e Raimundo Vicente de Sousa. Trata-se de uma versão preliminar, sendo que o conselho editorial poderá sugerir alterações para adequá-lo ao seu próprio estilo

ARTIGO 2 Blood serum parameters, meat quality and lipid profile of the meat from finishing pigs fed ractopamine in step up programs and two additional levels of lysine above basal requirements

Normas do Journal of Animal Sciences (versão submetida, sujeita a modificações)

The authors want to acknowledge the Brazilian National Council for Scientific and Technological Development (CNPq), to the CAPES Foundation and to the Research Support Foundation of Minas Gerais States (FAPEMIG) for financial support of this research. The first author thanks CAPES Foundation for the scholarship conceived for a sandwich exchange program at Purdue University – USA, scholar process 8944-11-3.

ABSTRACT: A trial was conducted in order to assess the effects of step up doses of ractopamine (RAC) and 2 levels of additional Lys above basal requirements fed to finishing pigs on biochemical parameters, meat quality and lipid profiles of the meat. Fifty-four barrows were blocked by initial BW (75.37 kg ± 2.88) and allotted to one out of nine treatments: negative control without addition of RAC nor Lys (NC), 7.5 ppm RAC constantly, 5 ppm RAC for 14 days followed by 10 ppm for 14 days (step up 1), 5 ppm RAC for 21 days followed by 10 ppm for 7 days (step up 2) and 5 ppm RAC for 7 days followed by 10 ppm for 21 days (step up 3). On constant and step up treatments 15 or 30% Lys above basal requirements were added. Experimental designed was a 4x2+1 factorial with six replicates. Blood from jugular vein was collected at slaughtering and loin muscle samples at the 10th rib were collected after 24 hours of refrigeration at 1°C. Glucose, high density lipoproteins (HDL-c), triacylglycerol (TG), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were evaluated through colorimetric kits. Color, dripping loss, cooking loss and Warner-Blatzer share force were assessed for meat quality analysis. Lipid profile of the meat was evaluated by gas chromatography. Programs with 15% additional Lys led to smaller concentration of serum HDL-c ($P < 0.01$). TG was not affected by any of the criteria evaluated ($P > 0.05$). RAC had a significant effect on AST ($P = 0.01$). No differences were found among treatments for any of the parameters tested regarding meat quality ($P > 0.05$). MUFA were influenced by Lys ($P = 0.05$) and RAC x Lys interaction ($P < 0.01$). S2-15 was 14.65% higher than S2-30 for MUFA ($P < 0.05$). RAC x Lys interaction was found for PUFA S2-30 was 42.73% higher than S2-15 (P

< 0.01). C16 desaturase levels were higher for programs with 15% additional Lys ($P < 0.01$). RAC did not influence on the blood parameters and pork from step up programs and 15% additional Lys kept standard quality and the lipid profile of the meat was not changed.

Key words: barrows, β-agonist, lysine, muscle, fat profile

INTRODUCTION

The idea that it is necessary to raise feed production in the world is well disclosed since the world's population was around 7.1 billion people in 2013 and predicted to be over 9 billion people in 2050 (PRB, 2013).

Ractopamine (RAC) is a β-adrenergic agonist (BAA) that has been extensively used in livestock production since its approval in US and other countries such as Australia, Brazil and Canada, aiming to ameliorate the efficiency of meat production by increasing the percentage of lean meat in the carcass (Bohrer et al., 2013). Besides leaner, the quality of meat must not be altered in order to achieve consumers' expectations.

RAC's mechanism of action leads to metabolic changes within the adipose and muscle cells (Halsey et al., 2011; Gunawan et al., 2007; Reiter et al., 2007), however, Weber et al. (2006) found little RAC effect on lipid profile of porcine tissues. Interestingly, Hoshi et al. (2005) found higher levels of triglycerides (TG), total cholesterol (TC) and high density lipids cholesterol (HDL-c) on pregnant sows fed RAC. BAA effects over

other serum parameters have been evaluated. Zilpaterol increased serum AST concentration in horses fed this BAA (Wagner et al., 2008), while Yaeger et al. (2012) fed RAC to dogs and found increased values on ALT and Chikhou et al. (1993) that observed increased values for AST as well as ALT in steers fed cimaterol.

The metabolic changes due to RAC feeding require additional Lys supplementation, which have been estimated through the years RAC have been used, some differences in these estimates were observed (NRC, 2012; Mitchell et al., 1990). Therefore, to test the hypothesis that RAC usage does not interfere on quality of pork, a study was designed to evaluate whether feeding step up RAC programs and two levels of Lys above basal requirements to finishing pigs interfere on serum parameters, meat quality and lipid profile of the meat.

MATERIAL AND METHODS

The procedures and housing adopted in this experiment were approved by the "Ethic Committee on Animal Use" of Federal University of Lavras, Lavras – Brazil.

Animals and housing

This experiment is part of a larger study that was conducted between November and December 2011 at the Swine Experimental Center of the Animal Science Department of Federal University of Lavras (UFLA), Lavras – Brazil, in which one hundred-eight finishing pigs (75.37 kg ± 2.88) from a high lean genetic line were used. For this study

fifth-four barrows we used. Pigs were housed two-by-two in concrete pens with free access to feed and water.

Treatments

Nine treatments were defined, been one negative control without RAC or Lys addition above basal levels. RAC was fed in step up programs as follow: step up 1 consisted on 5ppm RAC for 14 days followed by 10ppm RAC for 14 days, with 15 and 30% Lys (S1-15 and S1-30 respectively). Step up 2 consisted on 5ppm RAC for 21 days followed by 10ppm RAC for 7 days, with 15 and 30% Lys (S2-15 and S2-30 respectively). Step up 3 consisted on 5ppm RAC for 7 days followed by 10ppm RAC for 21 days, with 15 and 30% Lys (S3-15 and S3-30 respectively). Additionally, positive control treatments with 7.5ppm RAC with 15% or 30% Lys above basal levels (PC-15 and PC-30 respectively) were used throughout the experimental period.

Diets

All RAC diets were formulated to be isoproteic and isoenergetic to meet or exceed nutrition requirements according Rostagno et al. (2011). Corn and soybean oil levels varied between control diet and RAC diets in order to meet energy requirements. Lys levels were adjusted according to the treatments by adding amino acids to the feed keeping the amount of soybean and corn. Others essential amino acids were adjusted

according to Lys levels. Treatments with 30% Lys above basal levels needed an extra additional of tryptophan as well (Table 1).

Experimental design

The experiment was designed in a 4x2+1 augmented factorial design. Factors were four RAC programs and two levels of Lys supplementation above the basal level. The negative control treatment was used, thus representing the augmented factorial. The experiment had nine treatments and six replicates. The experimental unit was considered the pig.

Table 1. Experimental diets for finishing pigs fed ractopmine (RAC) on step up programs and two levels of lysine.

Ingredients (%)	Basal	15% lysine			30% lysine		
		5 ppm	7,5 ppm	10 ppm	5 ppm	7,5 ppm	10 ppm
Corn	82.000	75.500	75.500	75.500	75.500	75.500	75.500
Soybean meal, 46%	14.150	19.600	19.600	19.600	19.600	19.600	19.600
Soybean oil	0.570	1.900	1.900	1.900	1.900	1.900	1.900
Dicalcium phosphate	0.728	0.835	0.835	0.835	0.835	0.835	0.835
Calcium carbonate	0.590	0.620	0.620	0.620	0.620	0.620	0.620
Salt	0.329	0.330	0.330	0.330	0.330	0.330	0.330
Mineral and vitamin premix	0.500	0.500	0.500	0.500	0.500	0.500	0.500
DL-Methionine 99	0.018	0.033	0.033	0.033	0.068	0.068	0.068
L-Lysine 78	0.278	0.255	0.255	0.255	0.397	0.397	0.397
L-Threonine 98	0.058	0.063	0.063	0.063	0.139	0.139	0.139
Tryptophan 98	0.000	0.000	0.000	0.000	0.0155	0.0155	0.0155
Tylan G250®*	0.040	0.040	0.040	0.040	0.040	0.040	0.040
Clay	0.759	0.319	0.294	0.269	0.050	0.025	0.000
Ractosuin®**	0.000	0.025	0.050	0.075	0.025	0.050	0.075
Total	100.02	100.02	100.02	100.02	100.02	100.02	100.02
Metabolizable energy, Kcal/kg	3,230	3,300	3,300	3,300	3,300	3,300	3,300
Crude protein, %	13.00	15.00	15.00	15.00	15.00	15.00	15.00
Lysine, %	0.737	0.848	0.848	0.848	0.958	0.958	0.958
Methionine, %	0.228	0.263	0.263	0.263	0.297	0.297	0.297
Threonine, %	0.494	0.568	0.568	0.568	0.642	0.642	0.642
Tryptophan, %	0.13	0.153	0.153	0.153	0.172	0.172	0.172
Available phosphorus, %	0.226	0.25	0.25	0.25	0.25	0.25	0.25
Calcium, %	0.463	0.512	0.512	0.512	0.512	0.512	0.512

* Tilosin 250 g/Kg of product, Elanco Brasil, São Paulo, Brazil.
** Ractopamine 2%, Ourofino Saúde Animal, Cravinhos, Brazil.

Procedures

Pigs were allotted to pens and blocked by initial weight and had treatments randomly assigned. The pigs were fed the experimental diets *ad libitum* for 28 consecutive days. At the end of experimental pigs were fasted for 12 hours, weighed, and shipped to a commercial slaughter house running under Brazilian federal inspection and legislation (BRASIL, 2000). Blood samples were harvested from the jugular at the time of exsanguination for biochemical analysis of the blood.

After a four-hour resting period, pigs were taken to the restrainer for stunning and electrocution procedures, then bled and eviscerated. Carcasses were taken to the refrigerated room at approximately 2ºC.

Fragments sizing 10 x 10 cm with two cm thick from *Longissimus dorsi* muscle were harvested at the tenth rib from the left carcass, followed by freezing for posterior determination of physical and chemical characteristics of the meat.

Biochemical analysis of the blood

Blood was centrifuged for 10 min at 12.000 rpm in order to get the serum separated from the cell fraction. Glucose, cholesterol in high density lipoproteins (HDL-c), triacylglycerol (TG), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were evaluated through colorimetric kits (Gold Analisa Diagnóstica Ltda., Belo Horizonte – Brazil) following manufacture's recommendations using a TP-Analyzer Basic® reader (ThermoPlate, São Paulo, Brazil).

Meat laboratorial analysis

Analysis on meat samples were conducted at the Meat and Fish Laboratory of the Food Science Department of UFLA.

Measures of ultimate pH were taken from *Longissimus dorsi* at the tenth rib from the left side of the carcass, after 24 hours at approximately 2°C refrigeration. The muscle sample was sliced and the slice surface was exposed to the room ambient for 30 minutes. Color readings of luminosity (L*), red level (a*) and yellow level (b*) were taken on the slice surface using a Minolta CR-300® device (Minolta Corp., Ramsey, NJ) calibrated against white tile (Minolta calibration plate, Cr A 43). There were performed readings on three cuts of the same muscle at three different spots. Moreover, saturation index (C*) and tonality angle (h*) measures were also taken according the following formulae: C* = (a*2 + b*2)1/2 e; h* = tan-1 (b*/a*) (Ramos and Gomide, 2007).

Cooking loss (CL) was determined according AMASA (1978) using three slices of the muscle sample. Identified samples were weighed, wrapped in aluminum foil and cooked over a hot plate pre-heated at 150°C until reach internal temperature of 72 ± 2°C. After cooking samples were left refreshing until they attained room temperature and weighed again. Difference between initial weight and final weight correspond to CL. Samples utilized for CL were used for Warner-Bratzler shear force (WBSF) as well. Cooked samples were sliced in 2.0 x 1.0 x 1.0 cm pieces, such that the larger side was towards the longitudinal orientation of the muscle fibers according Froning & Uijttenboogarte (1988). The samples were then sliced transversely to the muscle fibers

using a Warner-Blatzler attached to a TA XT-2 texturometer (Stable Micro Systems, Surrey, UK).

To assess dripping loss (DL), muscle samples in duplicate were weighed and placed in plastic bags with several holes to allow dripping. Bags were kept inside inflated polyethylene bags and kept at 4°C for 24 hours and weighed. Difference between initial weight and final weight was the percentage of water loss accounted for dripping loss (Farouk & Price, 1994).

Lipid profile of the meat

Lipids were extracted according to Folch et al. (1957), esterified and separated according to Hartman and Lago (1973). Fatty acid (FA) analysis was performed using a Shimatzu CG 2010 (Agilent Technologies Inc., Palo Alto, CA, USA) gas-liquid chromatographer, equipped with flame ionization detector. Helium was injected at the rate of 1:100 using a 100-m SP-2560 Capillary GC Column™ (Supelco Chromatography Products, Bellefonte, PA). Initial temperature of the oven was 140ºC / 5 minutes followed by increasing of 4ºC / minute up to 240ºC, then and kept 240ºC for 30 more minutes. Injector and detector temperatures were 260ºC. FA were determined by comparing the retention times to those provided by the standard used (TM37 Component FAME Mix®, Supelco Inc., Bellefonte, PA, USA). FA concentrations were expressed in percentage relative to total FA. Then it was split into SFA, MUFA and PUFA. Enzyme activity of Δ-9 desaturase and elongase were estimated according to Malau-Aduli et al. (1998) and Kazala et al. (1999).

Atherogenicity and thrombogenicity indices, considered as a health indicator related to the risk of cardiovascular disease, were calculated according Ulbricht and Southgate (1991).

Statistical analysis

Data were analyzed and using Proc Mixed of SAS (SAS Inst., Inc., Cary, NC). Differences between treatment means and control treatments were tested by Duncan test for blood parameters and SNK test for muscle tissue and lipid profile analysis ($\alpha = 0.05$). Linear orthogonal contrasts were formulated to evaluate the effects of each aspect of the experiment. The negative control was first compared to the other treatments combined aiming determining whether RAC had effect on evaluation criteria; Lys addition effect (15 vs 30%) was analyzed as well; third contrast was formulated in order to compare the three step up treatments with 15% Lys accretion; forth contrast compared the three step up treatments with 30% Lys accretion; fifth contrast compared all the three step up programs with Lys accretion rates combined agains PC15 and PC30 combined; sixth contrast compared step up 1 vs step up 2; and seventh contrast compared step up 1 vs step up 3. It was considered 95% confidence level for all analysis. Not-normally distributed data were analyzed by Kruskal-Wallis test through SPSS statistical package.

RESULTS AND DISCUSSION

There were no Lys x RAC interactions ($P > 0.05$) for serum biochemical parameters or meat quality, and were therefore excluded from the model, allowing more reliable inferences once additional degrees of freedom were accounted for the error.

HDL-c, TG, GLU, AST and ALT means are presented in figure 1. Contrast analysis showed significant effects of RAC ($P < 0.01$), Lys ($P < 0.01$) and step up 15 ($P < 0.05$) for HDL-c (Table 2). Blood biochemical parameters are often very variable parameters, also considering it involves individual variation, even though the blood comes from a genetically controlled herd as well as controlled environment and feeding. In this study, coefficients of variation for blood biochemical parameters varied from 26.24 to 35.19%. Additionally, 15% Lys in the feed led overall to smaller concentrations of HDL-c in the blood. In fact, the group has conducted a metabolic study with the same animals, considering only the RAC-constant level treatments, and it was observed a slightly higher level of CPT-1, a lipolytic enzyme that drives the FA to the mithocondria for β-oxidation, on muscle tissue for PC15 compared to PC30 (8.2% higher, $P < 0.05$), which illustrates a possible higher index of lipolysis by those pigs (Ferreira et al., 2014, please refer to article 1).

Glucose means were different across treatments and notably varied ($P < 0.05$) between treatments. Glucose levels are influenced by several factors, one of which that is really relevant is fasting. Few researchers have investigated the effects of RAC on blood parameters. However, study demonstrated that RAC did not influence blood glucose

levels of pregnant sows fed 20 ppm RAC from the 25th day of pregnancy until the 80th day (Hoshi et al., 2005). On the other hand Araújo et al. (2014) fed increasing levels of RAC (0, 5, 10, 15 and 20 ppm) to finishing pigs and observed a linear effect of RAC on increasing blood glucose levels.

Table 2. Contrast analysis of biochemical parameters data of finishing pigs fed ractopamine (RAC) on Step up programs and two additional levels above basal values of lysine (n=54).

Item	P value of contrasts						
	RAC effect	LYS level effect	Lys step up 15 effect	Lys step up 30 effect	Step up effect	Step up 1 vs Step up 2	Step up 1 vs Step up 3
HDL-c	0.006	0.006	0.015	0.804	0.112	0.073	0.765
TG	0.386	0.116	0.203	0.888	0.420	0.946	0.620
ALT	0.985	0.160	0.554	0.063	0.358	0.578	0.862

HDL-c = high density lipoprotein; TG = triacylglycerol; ALT = alanine aminotransferase.

Hoshi et al. (2005) did not inform whether or not the animals were undergone fasting prior to each blood collection, indeed Araújo et al. (2013) did collect the blood after a twelve hours fasting due to Brazilian slaughter regulations. In this study pigs were fasted for 12 hours prior to slaughter due to federal inspection service regulation in Brazil, thus glucose levels might have been affected by the fasting period. The greatest difference between this study and Araújo et al. (2013) is the fact that these authors used different levels of RAC supplementation (0, 5, 10, 15 and 20 ppm) and did not compared each RAC inclusion level with the control, while this study was conducted using 5 or 7.5 ppm RAC and

treatments were also compared to control, therefore results across studies could not be compared.

TG blood levels were not different ($P > 0.05$) among treatments, except for S3-15 ($P < 0.05$). In a matter of fact, serum triglyceride levels are strongly influenced by fasting and, at a lesser extent by the diet. However in this study TG was not affected by any of the criteria evaluated in the contrast analysis ($P > 0.05$). Araújo et al. (2013) reported a linear increasing as the RAC level increases. Hoshi et al. (2005) and Kor et al. (2013) observed increased levels of TG, it is important to notice that Kor et al. (2013) used broiler chickens in their study. RAC had a significant effect on AST ($P = 0.01$). It might mean that there was a more intense amino acid metabolism, represented probably by RAC's effect of increasing protein synthesis.

Regarding physical and chemical characteristics of the meat, RAC and Lys did not interact ($P > 0.05$) for any of the parameters tested (Table 3). These results corroborate with previous reports in which RAC (Weber et al., 2006) and Lys (Madeira et al., 2013) did not have influence on physical characteristics of the meat in finishing hogs. Moreover, meat pH, C* and h* means did not present normal distribution ($P > 0.05$). Contrast outputs for chemical and physical parameters are presented on tables 4. No differences ($P > 0.05$) were found among treatments for any of the parameters tested regarding meat quality.

Previous researches have shown the absence of RAC effect on most of the meat quality evaluation criteria (Carr et al., 2005; Weber et al., 2006), however, Apple et al. (2008) used loin muscle samples to evaluate meat quality and observed a significant effect of RAC on

elevating the pH in 1,1%, but no effect on drip loss. Color objective measurements also pointed out to differences between RAC-fed pigs and control treatment (Weber et al., 2006; Carr et al., 2005).

Table 3. Meat quality evaluation criteria of finishing pigs fed ractopamine (RAC) and two levels of additional lysine above basal values for 28 days. (n=54).

Variable	NC	Additional lysine								SEM	*P* value		
		15%				30%							
		PC	S1	S2	S3	PC	S1	S2	S3		R	L	R*L
Ph	5.50	5.50	5.45	5.54	5.53	5.51	5.53	5.50	5.49	0.01	-	-	-
L*	53.59	54.19	53.31	53.04	53.77	54.21	53.75	53.97	52.87	0.23	0.67	0.81	0.66
a*	1.86	1.57	1.40	1.66	1.27	0.94	0.92	1.14	0.97	0.12	0.89	0.09	0.98
b*	12.37	12.06	12.06	11.92	12.05	12.06	12.00	12.09	11.58	0.10	0.87	0.69	0.79
C*	12.55	12.23	12.19	12.06	11.47	12.12	12.08	12.18	11.66	0.14	-	-	-
h*	81.70	76.27	83.65	82.36	84.36	85.76	85.93	81.68	85.36	1.01	-	-	-
DP	10.71	10.31	12.40	9.87	9.19	11.74	11.37	9.44	11.55	0.38	0.27	0.48	0.43
CL	22.62	20.83	23.53	23.66	23.27	20.21	23.34	20.97	22.32	0.36	0.06	0.16	0.68
WBSF	5.48	5.73	6.87	6.24	6.20	6.17	6.22	5.52	6.59	0.14	0.36	0.67	0.38

NC = Negative control, diet without RAC or additional lysine; PC = positive control, constant level of 7.5 ppm RAC; S1 = step up 1, 7 days of 5.0 ppm RAC followed by 21 days of 10 ppm; S2 = step up 2, 14 days of 5.0 ppm RAC followed by 14 days of 10 ppm; S3 = step up 3, 21 days of 5.0 ppm RAC followed by 7 days of 10 ppm.
L* = luminosity; a* = red level; b* = yellow level; C* saturation index; h* = tonality angle; DP = dripping loss; CL = cooking loss; WBSF = Warner-Bratzler shear force.

Although RAC helps on the control of the adiposity of finishing pigs, this assumption does not reflect on poorer meat quality. In a matter of fact, on past researches RAC had a little or no impact on meat quality (Apple et al., 2008; Weber et al., 2006), perhaps because the amount of fat present on the meat. In spite of the fact that fat deposition is slightly smaller for RAC-fed pigs, it still keeps color, marbling and firmness

unaltered (Weber et al., 2006). However, color measurements by subjective and objective methods may differ from study to study.

Table 4. Contrast analysis of chemical and physical evaluation criteria from finishing pigs fed ractopamine (RAC) on Step up programs and two additional levels above basal values of Lys (n=54).

Item	*P* value of contrasts						
	RAC effect	LYS level effect	Lys step up 15 effect	Lys step up 30 effect	Step up effect	Step up 1 vs Step up 2	Step up 1 vs Step up 3
L*	0.93	0.82	0.36	0.45	0.24	0.97	0.79
a*	0.10	0.06	0.77	0.87	0.92	0.50	0.91
b*	0.26	0.69	0.88	0.65	0.67	0.92	0.50
DP	0.98	0.50	0.90	0.50	0.70	0.07	0.22
CL	0.75	0.13	0.03	0.10	< 0.01	0.28	0.53
WBSF	0.11	0.65	0.15	0.90	0.35	0.12	0.72

L* = luminosity; a* = red level; b* = yellow level; DP = dripping loss; CL = cooking loss; WBSF = Warner-Bratzler shear force

Fatty acid profiles of the *Longissimus dorsi* were little or not influenced by RAC, however, they were influenced to a higher extent ($P < 0.05$) by Lys (Tables 5 and 6).

MUFA were influenced by Lys ($P = 0.05$) and RAC x Lys interaction ($P < 0.01$). Step 2 RAC programs responded in an opposite manner depending on Lys additional level, i.e., S2-15 was the RAC program with the higher level of MUFA among all treatments, and S2-30 was the lowest value. S2-15 was 14.65% higher than S2-30 ($P < 0.05$). In addition to that, RAC x Lys interaction was found for PUFA, which accounts for the difference observed between S2-15 and S2-30 ($P < 0.01$),

in which S2-30 was now 42.73% higher than S2-15. Moreover, the W6:W3 ratio was not influenced by RAC ($P > 0.05$).

In a human health stand point, it is known that the higher are the percentages of MUFA and PUFA, out of the total lipids in the meat, the better (Aronal et al., 2012). That been said, RAC did not have influence in the meat quality regarding fatty acid profiles, since it did not changed the amount of SFA, MUFA or PUFA. Additionally, Madeira et al. (2013) conducted a study with finishing pigs fed protein-restricted diets, adjusted and not adjusted for Lys, and they found no differences on SFA, MUFA neither PUFA between adjusted and not-adjusted Lys diets, which means that the Lys percentage did not influence on those parameters. Levels of ω-6 were different, S2-30 led to a higher level of this fatty acid compared to the other treatments ($P < 0.01$).

All ω-6 fatty acids evaluated are PUFA, what explains mathematically the higher levels of PUFA for S2-30. It corroborates with Weber et al. (2006) and Apple et al. (2008) whom found no RAC effects on fatty acid profiles. It can be noticed that C16 desaturase activity, one of the enzymes responsible for desaturation of fatty acids, was influenced by RAC, Lys and their interaction ($P < 0.01$), pigs fed 15% additional Lys had 7.5 % higher ($P < 0.01$) C16 desaturase than 30%. Elongase and thioesterase activities were also influenced by RAC x Lys ($P < 0.01$ and $P < 0.05$, respectively), meanwhile atheriogenicy index was influenced by RAC, Lys and the interaction ($P < 0.01$), however there is no trend for these alterations, such that the authors can affirm whether RAC or Lys levels could lead to healthier meat for human consumption.

Table 5. Fatty acid profiles of the meat (*Longissimus dorsi*) muscle from finishing pigs fed ractopmine (RAC) and two additional levels of lysine for 28 days.

Variable	NC	Additional lysine								SEM	[1]*P* value		
		15%				30%							
		PC	S1	S2	S3	PC	S1	S2	S3		R	L	R*L
C14:0	1.19	1.10	1.32	1.28	1.21	1.29	1.32	1.04	1.26	0.03	0.30	0.95	0.09
C14:1	0.08	0.16	0.08	0.04	0.06	0.12	0.19	0.08	0.22	0.03	-	-	-
C15:0	0.08	0.10	0.07	0.03	0.08	0.11	0.17	0.13	0.10	0.01	-	-	-
C16:0	25.81	25.72	26.30	25.62	25.32	24.42	25.61	25.85	25.41	0.18	0.36	0.27	0.45
C16:1	2.35 AB	2.68 BC	3.01 C	2.87 C	2.38 AB	2.20 A	2.60 ABC	2.27 AB	2.82 C	0.06	0.11	0.01	< 0.01
C17:0	0.42 a	0.46 a	0.26 bc	0.19 c	0.35 ab	0.41 a	0.37 ab	0.41 a	0.49 a	0.02	-	-	-
C17:1	0.13	0.03	0.08	0.15	0.23	0.18	0.11	0.10	0.11	0.02	-	-	-
C18:0	12.56	12.55	11.86	11.56	12.54	13.16	12.30	12.52	12.43	0.15	0.25	0.15	0.69
C18:1ω9t	0.03	0.02	0.06	0.09	0.10	0.23	0.06	0.01	0.11	0.02	-	-	-
C18:1ω9c	42.63 BC	42.50 BC	42.75 BC	45.71 A	43.13 B	43.30 B	43.55 B	40.09 C	42.74 BC	0.33	0.99	0.06	< 0.01
C18:2ω6c	11.88 B	11.97 B	11.65 B	10.49 B	11.58 B	11.61 B	11.04 B	14.30 A	11.35 B	0.25	0.41	0.18	< 0.01
C20:0	0.07	0.06	0.09	0.14	0.17	0.18	0.15	0.15	0.08	0.01	-	-	-
C18:3ω6	0.04 a	0.02 b	0.00 c	0.00 c	0.04 a	0.03 ab	0.10 a	0.14 a	0.01 b	0.01	-	-	-

Table 5. Fatty acid profiles of the meat (*Longissimus dorsi*) muscle from finishing pigs fed ractopmine (RAC) and two additional levels of lysine for 28 days (Continuation).

Variable	NC	Additional lysine								SEM	[1]*P* value		
		15%				30%							
		PC	S1	S2	S3	PC	S1	S2	S3		R	L	R*L
C20:1	0.53	0.51	0.60	0.52	0.61	0.57	0.63	0.52	0.66	0.02	0.37	0.53	0.98
C18:3ω3	0.35	0.26	0.32	0.26	0.28	0.29	0.31	0.21	0.34	0.02	0.63	0.91	0.87
C20:2	0.25	0.24	0.27	0.22	0.17	0.30	0.21	0.26	0.29	0.02	-	-	-
C20:3ω6	0.18	0.14	0.15	0.08	0.22	0.19	0.11	0.12	0.09	0.02	-	-	-
C20:4ω6	1.39 AB	1.46 AB	1.10 AB	0.72 B	1.51 AB	1.41 AB	1.19 AB	1.77 A	1.47 AB	0.07	0.33	0.07	0.03
[a]SFA	40.14	40.00	39.90	38.84	39.68	39.58	39.91	40.12	39.78	0.27	0.97	0.69	0.78
[b]MUFA	45.76 B	45.90 B	6.59 B	49.39 A	46.52 B	46.60 B	47.13 B	43.08 C	46.67 B	0.35	0.86	0.05	< 0.01
[c]PUFA	14.10 AB	14.10 AB	13.50 AB	11.77 B	13.80 AB	13.82 AB	12.96 B	16.80 A	13.55 AB	0.32	0.65	0.11	< 0.01
[d]Σω3	0.35	0.26	0.32	0.26	0.28	0.29	0.31	0.21	0.34	0.02	0.63	0.91	0.87
[e]Σω6	13.50 B	13.60 B	12.90 B	11.29 B	13.35 B	13.23 B	12.44 B	16.32 A	12.91 B	0.32	0.57	0.11	< 0.01
[f]Σω6/Σω3	45.75	68.79	48.06	47.10	34.55	36.38	40.66	66.29	42.19	4.27	0.69	0.93	0.15
[g]PUFA/SFA	0.35	0.36	0.34	0.31	0.35	0.35	0.32	0.42	0.34	< 0.01	0.67	0.22	0.06
[h]Δ9-des^{C16}	8.29 BC	9.45 AB	10.24 A	10.10 A	8.59 BC	8.29 BC	9.16 ABC	8.07 C	9.98 A	0.18	< 0.01	< 0.01	< 0.01

Table 5. Fatty acid profiles of the meat (*Longissimus dorsi*) muscle from finishing pigs fed ractopmine (RAC) and two additional levels of lysine for 28 days (Continuation).

Variable	NC	Additional lysine								SEM	[1]*P* value		
		15%				30%							
		PC	S1	S2	S3	PC	S1	S2	S3		R	L	R*L
[i]Δ9-des^{C18}	77.21	77.20	78.23	79.83	77.45	76.65	77.97	76.19	77.46	0.30	0.58	0.16	0.06
[j]ELO$^{C16-C18}$	66.21 AB	65.97 AB	65.07 B	66.78 AB	66.76 AB	67.96 A	66.44 AB	65.17 B	66.14 AB	0.22	0.23	0.75	< 0.01
[k]THES^{C16-14}	95.60	95.89	95.22	95.22	95.42	94.95	95.15	96.13	95.27	0.11	0.53	0.79	0.02
[l]ATG	0.56 BC	0.55 C	0.59 AB	0.60 A	0.56 BC	0.55 C	0.58 ABC	0.53 D	0.57 BC	< 0.01	< 0.01	< 0.01	< 0.01
[m]TRBG	1.29	1.29	1.29	1.24	1.27	1.27	1.28	1.30	1.27	0.01	-	-	-

[1] R, L, R*L: effects of RAC, lysine and their interaction, respectively; [a]Total saturated fatty acids, SFA (C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0); [b]Total monounsaturated fatty acids, MUFA (C14:1 cis-9 + C16:1 cis-9 + C17:1 cis-9 + C18:1 cis-9+ C18:1 trans-9 + C20:1 cis-9); [c]Total polyunsaturated fatty acids, PUFA (C18:2ω-6 + C18:3ω-6 + C18:3ω-3 + C20:4ω-6 + C20:3ω6); [d] Total PUFA from n-3 series (C18:3ω-3); Total PUFA from n-6 series (C18:2ω-6 + C18:3ω-6 + C20:4ω-6); [f] Ratio ω-3/ω-6; [g] Ratio PUFA/SFA; [h]C16 desaturase activity = 100 [(C16:1 cis-9)/(C16:1 cis-9+C16:0)]; [i] C18 desaturase activity index=100 [(C18:1 cis-9)/(C18:1 cis-9+C18:0)]; [j]C16 to C18 elongase activity = 100 [(C18:0+C18:1 cis-9)/(C16:0+C16:1cis-9+C18:0+C18:1 cis-9)]; [k]C16 to C14 thioesterase activity = 100 [(C16:0)/(C16:0+C14:0)]; [l]Atherogenicity index, ATG = [4(C14:0) + C16:0]/(Sum SFA + Sum PUFA); [m]Thrombogenicity index, TRBG = (C14:0 + C16:0 +C18:0) / [(0.5xΣMUFA) + (0.5xΣω6) + (3xΣω-3) + (Σω-3/ω-6)]. Means not sharing same capital letter in row differ by SNK test (α = 0.05). Means not sharing same small letter in a row differ by Kruskal-Wallis test (α = 0.05).

Table 6. Contrast analysis of fatty acid profiles of the meat (*Longissimus dorsi*) evaluation criteria from finishing pigs fed ractopamine (RAC) on Step up programs and two additional levels above basal values of lysine.

Item	*P* value of contrasts						
	RAC effect	LYS level effect	Lys step up 15 effect	Lys step up 30 effect	Step up effect	Step up 1 vs Step up 2	Step up 1 vs Step up 3
C14:0	0.71	0.95	0.11	0.39	0.60	0.09	0.37
C16:0	0.65	0.30	0.97	0.07	0.19	0.70	0.30
C16:1	0.04	< 0.01	0.58	0.01	0.02	0.05	0.08
C18:0	0.70	0.17	0.31	0.18	0.10	0.94	0.39
C18:1ω9c	0.63	0.03	0.09	0.14	0.86	0.71	0.75
C18:2ω6c	0.83	0.12	0.28	0.36	0.90	0.08	0.84
C20:1	0.63	0.58	0.48	0.741	0.46	0.28	0.83
C18:3ω3	0.38	0.91	0.69	0.96	0.80	0.27	0.93
C20:4ω6	0.72	0.04	0.09	0.73	0.33	0.57	0.06
[a]SFA	0.70	0.73	0.65	0.76	0.92	0.67	0.86
[b]MUFA	0.27	0.01	0.03	0.18	0.53	0.31	0.66
[c]PUFA	0.70	0.07	0.23	0.49	0.71	0.17	0.56
[d]Σω3	0.38	0.91	0.69	0.96	0.80	0.27	0.93
[e]Σω6	0.74	0.06	0.18	0.40	0.71	0.11	0.50
[f]Σω6/Σω3	0.87	0.73	0.11	0.40	0.58	0.37	0.66
[g]PUFA/SFA	0.92	0.22	0.40	0.75	0.71	0.24	0.63
[h]Δ9-des^{C16}	< 0.01	< 0.01	0.58	0.03	0.05	0.04	0.17
[i]Δ9-des^{C18}	0.64	0.06	0.17	0.56	0.17	0.91	0.43
[j]ELO$^{C16-C18}$	0.89	0.47	0.71	< 0.01	0.05	0.68	0.20
[k]THES^{C16-14}	0.54	0.77	0.09	0.11	0.95	0.11	0.58

Table 6. Contrast analysis of fatty acid profiles of the meat (*Longissimus dorsi*) evaluation criteria from finishing pigs fed ractopamine (RAC) on Step up programs and two additional levels above basal values of lysine (Continuation).

Item	*P* value of contrasts						
	RAC effect	LYS level effect	Lys step up 15 effect	Lys step up 30 effect	Step up effect	Step up 1 vs Step up 2	Step up 1 vs Step up 3
[l]ATG	0.47	< 0.01	< 0.01	0.52	0.01	0.01	0.02

[a]Total saturated fatty acids, SFA (C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0); [b]Total monounsaturated fatty acids, MUFA (C14:1 cis-9 + C16:1 cis-9 + C17:1 cis-9 + C18:1 cis-9+ C18:1 trans-9 + C20:1 cis-9); [c]Total polyunsaturated fatty acids, PUFA (C18:2ω-6 + C18:3ω-6 + C18:3ω-3 + C20:4ω-6 + C20:3ω6); [d] Total PUFA from n-3 series (C18:3ω-3); Total PUFA from n-6 series (C18:2ω-6 + C18:3ω-6 + C20:4ω-6); [f] Ratio ω-6/ω-3; [g] Ratio PUFA/SFA; [h]C16 desaturase activity = 100 [(C16:1 cis-9)/(C16:1 cis-9+C16:0)]; [i] C18 desaturase activity index=100 [(C18:1 cis-9)/(C18:1 cis-9+C18:0)]; [j]C16 to C18 elongase activity = 100 [(C18:0+C18:1 cis-9)/(C16:0+C16:1cis-9+C18:0+C18:1 cis-9)]; [k]C16 to C14 thioesterase activity = 100 [(C16:0)/(C16:0+C14:0)]; [l]Atherogenicity index, ATG = [4(C14:0) + C16:0]/(Sum SFA + Sum PUFA).

Lipid source indeed influence the fatty acid profiles of the meat in pigs (Sousa et al., 2010, Weber et al., 2006), however, according to the findings in this study, as well as previous research discussed previously, neither RAC nor Lys have this ability. The main goal of this study was to test the hypothesis that RAC usage did not mitigate the quality of the meat in pigs fed step up doses of RAC associated to 2 levels of additional Lys beyond the basal requirements. In addition to that the study was conducted to verify whether a slight reduction on the additional Lys level required by RAC would play a role on meat quality and lipid profile of the meat. As the results in this study did not show any interference, we can thus affirm that Lys addition on 15% above basal levels can be used by producers since crystalized amino acids consist in expensive ingredients of animal diets.

Conclusions

RAC did not influence on blood parameters tested. Additionally, pork from finishing pigs fed RAC on step up programs and 15% additional Lys kept standard quality and the lipid profile of the meat was not changed.

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Figure 1

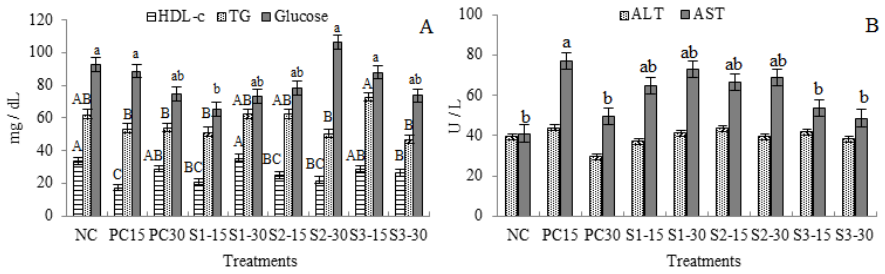

Figure 1. Blood biochemical parameters of finishing barrows fed ractopamine for 28 days. Means not sharing same capital letter differ by SNK test (α = 0.05). Means not sharing same small letter differ by Kruskal-Wallis test (α = 0.05). HDL-c = high density lipoprotein; TG = triacylglycerol; ALT = alanine aminotransferase; AST = aspartate aminotransferase.

Artigo redigido em co-autoria com Vivian de Oliveira Silva, Peter T. Faria, Vinícius de Souza Cantarelli e Raimundo Vicente de Sousa. Trata-se de uma versão preliminar, sendo que o conselho editorial poderá sugerir alterações para adequá-lo ao seu próprio estilo.