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UNIVERSIDADE ESTADUAL DE CAMPINAS
FACULDADE DE ENGENHARIA DE ALIMENTOS
DEPARTAMENTO DE TECNOLOGIA DE ALIMENTOS

RAQUEL FORMIGHIERI

EFEITO DA RACTOPAMINA E DA IMUNOCASTRAÇÃO NO BEM-ESTAR ANIMAL E NAS PROPRIEDADES DA CARNE SUÍNA

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DISSERTAÇÃO DE MESTRADO APRESENTADA À FACULDADE DE ENGENHARIA DE ALIMENTOS UNICAMP PARA OBTENÇÃO DO TÍTULO DE MESTRE EM TECNOLOGIA DE ALIMENTOS

PROF. DR. PEDRO EDUARDO DE FELÍCIO/ORIENTADOR

DR. EXPEDITO TADEU FACCO SILVEIRA/CO-ORIENTADOR

Este exemplar corresponde à versão final da dissertação defendida por Raquel Formighieri, aprovada pela comissão julgadora em ___/___/___ e orientada pelo Prof. Dr. Pedro Eduardo de Felício

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SUMÁRIO

RESUMO GERAL

A adição de ractopamina (RAC) na dieta de suínos em terminação tem sido amplamente utilizada para melhorar o desempenho zootécnico, porém tem despertado questionamentos no âmbito do bem-estar animal e qualidade da carne suína. Além disso, a posibilidade de ocorrer resíduos dessa substância na carne preocupa alguns países e a União Européia. Os objetivos desse trabalho são avaliar os possíveis efeitos da combinação da ractopamina e imunocastração, nas propriedades da carne e nas condições físicas e fisiológicas de bem-estar dos suínos. Um total de 310 fêmeas, machos castrados e imunocastrados de duas granjas diferentes e distintos cruzamentos genéticos [Tempo macho × Topigs 40 fêmea, granja A (n = 202) e AGPIC 337 macho × CB 22 fêmeas da granja B (n = 108)] foram aleatoriamente designados para receber o tratamento com RAC (7,5 mg / kg), durante 21 (±2) dias antes do abate, ou a dieta convencional, sem RAC. Na granja A, vocalizações foram registradas, para cada tratamento, durante a condução dos animais para o *box* de atordoamento. Os 202 suínos provenientes da granja A e os 108 suínos da granja B foram abatidos em diferentes abatedouros comerciais e as carcaças foram resfriadas por 24 horas a 4°C. Amostras de sangue foram coletadas na sangria, para determinar a atividade enzimática da creatina-fosfoquinase (CPK), lactato desidrogenase (LDH) e os níveis do hormônio cortisol. As patas dianteiras foram avaliadas, após o abate, para contabilizar lesões de três categorias distintas: rachadura, erosão e hematoma. Após escaldagem e evisceração, as carcaças foram avaliadas para o desenvolvimento precoce de *rigor mortis* e escoriações de pele. Antes do refriamento, 60 carcaças de cada granja foram selecionadas para avaliações das propriedades da carne. Após 24 horas na câmara de resfriamento, o pH_{24h} foi determinado no músculo *longissimus dorsi* (LD) e no músculo *semimembranosus* (SM) das carcaças, enquanto que a cor objetiva, perdas por exsudação, perdas por cozimento e força de cisalhamento (WBSF) foram medidos somente nas amostras do LD. Não

houve interações em nenhuma das propriedades da carne estudada ($P \geq 0,06$). A ausência de interação entre condição sexual e ractopamina, sugere que a combinação de RAC e imunocastração não influencia a qualidade da carne. A incidência de RSE (Red, Soft, Exudative – carne vermelha, flácida e exsudativa) foi extremamente elevada, 80,0 % do total das amostras avaliadas apresentaram esse problema de exsudação e 10,3% foram classificadas como PSE (Pale, Soft, Exudative – carne pálida, flácida e exsudativa) e somente 0,9 % apresentaram carne DFD (Dark, Firm, Dry – escura, firme e seca), mas a RAC não influenciou essas incidências. A adição de RAC em 7,5 mg/kg na dieta de suínos em terminação não apresentou impacto importante sobre os índices sanguíneos de estresse (CPK e LDH), lesões de casco e medidas da carcaça. No entanto, fêmeas alimentadas com RAC apresentaram maiores valores de cortisol. Alguns parâmetros de vocalização, como a intensidade do som e amplitude também foram maiores para os animais alimentados com RAC. Em relação às propriedades da carne, a dieta com RAC elevou perdas por cocção e valores de força de cisalhamento. Em relação à condição sexual, de uma forma geral as fêmeas apresentaram valores menores para os índices de estresse avaliados em comparação com imunocastrados e castrados cirurgicamente, com exceção para as medições dos níveis de cortisol, onde as fêmeas alimentadas com RAC apresentaram a maior concentração. É difícil apontar qual categoria de sexo foi mais susceptível ao estresse, uma vez que os parâmetros avaliados apresentaram resultados de forma equilibrada ente castrados e imunocastrados. A condição sexual não apresentou impacto importante sobre as propriedades da carne suína; imunocastrados não diferiram de castrados cirurgicamente. A diferença entre as granjas onde os animais foram criados influenciou vários parâmetros de bem-estar animal e também algumas propriedades da carne suína. Animais criados na granja A apresentaram maior incidência de índices de estresse negativos e valores superiores em algumas propriedades da carne, como: pH_{24} (SM), cor objetiva (a*), perdas por cocção, força de cisalhamento, além de maior ocorrência de lesões de casco (hematomas e rachaduras), desenvolvimento precoce de *rigor mortis* e aumento nos níveis sanguíneos de LDH, quando comparados a animais criados na granja B. Estas diferenças podem estar associadas a certos fatores, como estrutura da granja e abatedouro, transporte, manejo, contato com humanos e principalmente a origem do cruzamento genético dos animais.

ABSTRACT

The addition of ractopamine (RAC) to the diet of finishing pigs has been widely used to improve production performance; however it has raised questions in the fields of animal welfare and pork quality. In addtion, the possibility of residues of this substance occurs in the meat leads to concern some countries and the European Union. The objectives of this study were to examine the effects of RAC and imunocastration combined on pork properties and the physical and physiological conditions of welfare in pigs. A total of 310 gilts, immunocastrates and barrows from two different farms and distinct genetic crosses [Tempo sires × Topigs 40 dams from farm A (n=202) and AGPIC 337 sires × CB 22 dams from farm B (n = 108)] were randomly assigned to received the RAC treatment (7.5 mg/kg), 21 (±2) days prior slaughter, or the conventional diet without RAC. In farm A, vocalizations were recorded, for each treatment within sex condition, during the animals' conduction to the stunning box. The 202 pigs from farm A and the 108 pigs from farm B, were slaughtered at different commercial slaughterhouses and chilled for 24 h at 4°C. Blood samples were collected at exsanguination to determine the enzymatic activity of creatine phosphokinase (CPK), lactate dehydrogenase (LDH) and the levels of the hormone cortisol. Front hooves were accessed; post slaughter, to evaluate 3 distinct lesions categories: splits, crack-erosions and bruises. After scalding and evisceration, carcasses were evaluated for early rigor development and skin damage score. Before chilling, 60 carcasses from each farm were selected for pork quality. After 24 h in the chiller, the pH_{24h} was determined on the M. *longissimus dorsi* (LD) and on the M. *semimembranosus* (SM) of carcasses, whereas the objective color, drip loss, cooking loss and Warner-Bratzler shear force (WBSF) were measured on the LD samples only. No interaction for any of the meat properties studied were detected ($P \geq 0.06$). The absence of sex x RAC interaction, suggests that the combination of RAC and immunocastration had no impact on pork quality. The

incidence of RSE – red, soft and exudative - was extremely high, 80% of the total samples evaluated had this exudation problem, 10.3 % were classified as PSE - pale, soft and exudative, and 0.9 % were classified as DFD – dark, firm and dry. The addition of RAC at 7.5 mg/kg on the diet of finishing pigs did not impact blood parameters of stress (CPK and LDH), hoof lesions and carcass measurements. However, some vocalization parameters and cortisol for females were greater for RAC-fed pigs. PSE and RSE meat was not increased by RAC diet, however cooking loss and WBSF toughness were increased in samples from RAC-fed pigs. In general, gilts presented lower values for the indices of stress than immunocastrates or barrows, with the exception of measurements of cortisol levels, where RAC-fed gilts had the highest concentrations. It is difficult to point wich sex category was more susceptible to stress, once different results were observed for each sex in different stress parameters measured. Animal sex condition had no important impact on fresh pork properties; immunocastrated pigs did not differ from barrows. The farms and processing plants influence the incidence of several welfare parameters and pork properties. Animals from farm A presented greater indices of stress and pork properties, including pH_{24}, a* values, cooking loss, shear force, splits, bruises, early development of *rigor mortis* and LDH blood levels, than farm B. This difference can be associated to some factors, such as farm and slaughterhouse facilities, transport, humane handling and mainly genetic background.

INTRODUÇÃO GERAL

A carne suína é a mais consumida no mundo, fornecendo cerca de 38% da ingestão protéica diária mundial, embora seu consumo varie amplamente de lugar para lugar, em função de hábitos, proibições religiosas ou dogmáticas (ABIPECS, 2010). O Brasil é apontado como o país que poderá liderar a produção mundial de suínos por ser um dos maiores produtores de grãos, condição primária para a sustentação da cadeia suinícola (BRIDI et al., 2006), além de possuir clima apropriado, grandes extensões territoriais e mão-de-obra abundante. As expectativas de atendimento deste mercado exigem investimento contínuo para melhorar a produtividade e a qualidade da carne.

Em 1970, o plantel de suínos era de 31,5 milhões de cabeças e a produção havia sido de 705 mil toneladas. Em 2005, com 32,9 milhões de cabeças, a produção aumentou para 2,707 milhões de toneladas. Portanto, em 35 anos, o crescimento do plantel foi de apenas 4,4% enquanto que a produção de carne aumentou 283%. Esses dados mostram o salto tecnológico dado pelo setor suinícola em pouco mais de três décadas, em virtude de um forte investimento em seleção genética, manejo e nutrição, os quais refletem grandes avanços na maior eficiência da produção animal (ROPPA, 2006).

No âmbito da nutrição animal, diversas estratégias tecnológicas têm sido utilizadas. Entre os recursos nutricionais, a inclusão de cloridrato de ractopamina (RAC) em dietas de suínos em terminação tem demonstrado melhorar o desempenho animal. A RAC age como agente repartidor, redirecionando os nutrientes para aumentar a síntese proteíca, ao invés de depositar gordura (RICKS et al., 1984; MOODY et al., 2000; GONZALES et al., 2010). RAC é estruturalmente semelhante aos hormônios naturais norepinefrina e epinefrina e pode ser utilizado para melhorar a eficiência alimentar (UTTARO et al., 1993; SEE et al., 2004; WEBER et al., 2006; MARINHO et al., 2007). O uso da RAC foi provado melhorar a taxa de crescimento, conversão alimentar

(WILLIAMS et al., 1994; STOLLER et al., 2003), ganho de peso diário (SEE et al., 2004) e produzir carcaças mais magras (WEBER et al., 2006). Nos Estados Unidos, a ractopamina é utilizada desde 1999, ano em que foi aprovada pelo Food and Drug Administration (FDA), no Brasil essa substância foi aprovada em 2001 e desde então tem sido amplamente utilizada na dieta de suínos em terminação. Apesar dos efeitos positivos da RAC sobre o desempenho de produção animal estarem bem estabelecidos, é preciso ficar atento às possíveis alterações na qualidade da carne e mudanças no comportamento e bem-estar animal.

O método de castração cirúrgica é uma prática comumente realizada com o principal objetivo de prevenir a ocorrência de odor sexual na carne de suínos (EVANS, 2006). Países como a Espanha, abatem animais antes da maturidade sexual, a fim de evitar a deposição dos compostos responsáveis pelo odor sexual na carne. No Brasil, a legislação não permite o abate de suínos machos inteiros para fins comerciais (BRASIL, 1952; BRASIL, 1988). Dessa forma, surge na Austrália em 1998 um método alternativo de inibição temporária dos compostos sexuais e redução do acúmulo de ferormônios na gordura das carcaças, a imunocastração. No Brasil, a castração imunológica somente foi aprovada para uso comercial em 2007.

A castração imunológica é realizada através da aplicação de duas doses, 4 e 8 semanas antes do abate, da vacina Vivax® a qual produz anticorpos contra o fator de liberação de gonadotrofinas (GnRF), o que resulta em diminuição dos hormônios sexuais na corrente sanguínea. Essa tecnologia proporciona significativo ganho no bem-estar animal, uma vez que substitui o cruento método de castração cirúrgica, além de aproveitar os benefícios dos efeitos dos esteróides testiculares no crescimento e desenvolvimento da carcaça durante a fase que antecede a aplicação da segunda dose da vacina (DUNSHEA et al., 2001; OLIVER et al., 2003).

Um número limitado de pesquisas está disponível sobre a utilização dessas tecnologias combinadas. Assim, o objetivo deste estudo é avaliar os efeitos da ractopamina em suínos imunocastrados, castrados cirurgicamente e fêmeas, no que diz respeito à susceptibilidade ao estresse e possíveis alterações nas propriedades da carne fresca.

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REVISÃO DE LITERATURA

1. AGONISTAS β-ADRENÉRGICOS

Agonistas β-adrenérgicos têm sido estudados por décadas na produção animal com o intuito de melhorar a produtividade. Cunningham et al. (1963) observaram que a epinefrina poderia aumentar o ganho de peso diário e a retenção de nitrogênio em suínos, entretanto parece ter havido uma diminuição no interesse pela ação destas substâncias, sendo somente retomado na década de 80, quando diversos agonistas β-adrenérgicos foram desenvolvidos. Existem pelo menos seis agonistas β-adrenérgicos que tem mostrado aumentar a síntese proteíca na carcaça de animais: ractopamina, cimaterol, L-644,969, isoproterenol, salbutamol e clenbuterol, zilpaterol (WRAY-CAHEN, 2001).

2. RACTOPAMINA

O cloridrato de ractopamina (RAC) é um agonista β-adrenérgico classificado como uma fenetanolamina, análogo estrutural das catecolaminas, epinefrina e norepinefrina, é caracterizada pela presença de anel aromático, cadeia lateral da etanolamina e o nitrogênio alifático (Smith, 1998). Atua como modificador do metabolismo do animal e age como agente repartidor de nutrientes, pois tem a capacidade de redirecionar a distribuição normal de nutrientes em função do metabolismo da célula. Desta forma, faz com que nutrientes utilizados para produção do tecido adiposo sejam usados para aumento da síntese proteíca (RICKS et al., 1984; WATKINS et al., 1990; MOODY et al., 2000).

2.1 MECANISMO DE AÇÃO

A ractopamina e constiuída por dois carbonos quirais ligados a quatro estereoisômeros, RR, RS, SR, SS (Ricke et al., 1999). O isômero RR parece ser o isômero ativo, pois tem maior afinidade e maior eficácia para a ativação da adenilil ciclase através dos receptores β dos suínos. A ordem de classificação de afinidades para cada subtipo é RR> RS > SR> SS (MILLS et al., 2002).

Agonistas β-adrenérgicos atuam nas células via receptores das membranas (WARRISS, 1989). Os receptores de catecolaminas são classificados como α, β1 e β2 adrenérgicos, de acordo com o padrão de suas respostas e local de ligação. Deste modo, α receptores são associados com a vasoconstrição do útero e muscúlo liso, β1 com o aumento dos batimentos cardíacos, lipólise e glicogenólise e β2 receptores com o relaxamento dos brônquios, traquéias, musculatura lisa vascular e útero; e com contração do músculo estriado (LAWRIE e LEDWARD, 2006).

Apesar de o efeito da RAC no metabolismo animal ser causado pela ativação de β receptores, ainda não está bem esclarecido quais deles são efetivamente ativados. A maioria dos autores afirma que a RAC age predominantemente nos receptores β1 (MOODY et al., 2000; ZAGURY, 2002; SEE et al., 2004; LAWRIE e LEDWARD, 2006), outros nos β2 recepetores (RAMOS e SILVEIRA, 2002) e alguns referem-se somente a β receptores (MARCHANT-FORDE et al., 2003; BRIDI et al., 2006; STRYDOM et al., 2009). Entretanto, segundo estudo realizado por Mills et al., (2002) a ractopamina atua em ambos receptores, porém o isômero RR possue maior atividade agonista pelo receptor β2 na membrana dos suínos (MILLS et al., 2003).

Em suínos, os receptores β3 são detectados em vários tecidos, mas representam menos de 2% dos subtipos presentes em todos os tecidos, com exceção do tecido adiposo. Os receptores β1 são predominantes na maioria dos tecidos, atingindo quase 80% no tecido adiposo, 72% no coração, 65% nos pulmões, 60% no músculo esquelético e 50% no fígado como mostrado na Tabela 1 (MCNEEL e MERSMANN, 1999).

Tabela 1: Porcentagem de cada subtipo de β receptor em diversos tecidos de suínos (MCNEEL e MERSMANN, 1999).

Tecido	β1	β2	β3
Coração	72	28	0,25
Pulmão	67	33	0,2
Fígado	45	55	0
Músculo Esquelético	60	39	0,7
Tecido adiposo	73	20	7

2.2 AÇÃO NO TECIDO ADIPOSO E MUSCULAR

.A diminuição da deposição lipídica nas carcaças de animais alimentados com agonistas β-adrenérgicos é consequência da direta ativação dos β receptores nos adipócitos que promovem hidrólise e diminuição na síntese de lipídios (MILLS et al., 2002). Porém, o mecanismo bioquímico de ação da RAC na célula ainda não está bem esclarecido. Entretanto, sabe-se que agonistas β-adrenérgicos ligam-se a β receptores específicos na membrana celular, o que causa uma mudança conformacional no receptor. Este mecanismo permite que o complexo agonista β-adrenérgico-receptor se una a uma proteína reguladora guanosina trifosfato (GTP), causando uma alteração de conformação da proteína G, que permite que a GTP se transforme em guanosina difosfato (GDP). A GTP ativa a proteína G e interage com adenilato ciclase, formando um complexo catalítico ativado que converte adenosina trifosfato (ATP) para adenosina monofosfato cíclica (AMPc). A adenosina monofosfato cíclica age sobre proteínas quinase-dependentes de AMP cíclico. O AMPc liga-se à subunidade da proteína quinase A (PKA), ativando-a. A PKA, por sua vez, libera suas subunidades catalíticas causando a fosforilação de várias enzimas que aumentam a taxa de lipólise e reduzem o anabolismo, como pode ser observado na Figura 1. A atividade catalítica é determinada pela hidrólise de GTP em GDP (Figura 1) (CONVEY et al., 1987; MOODY et al., 2000; MILLS, 2002).

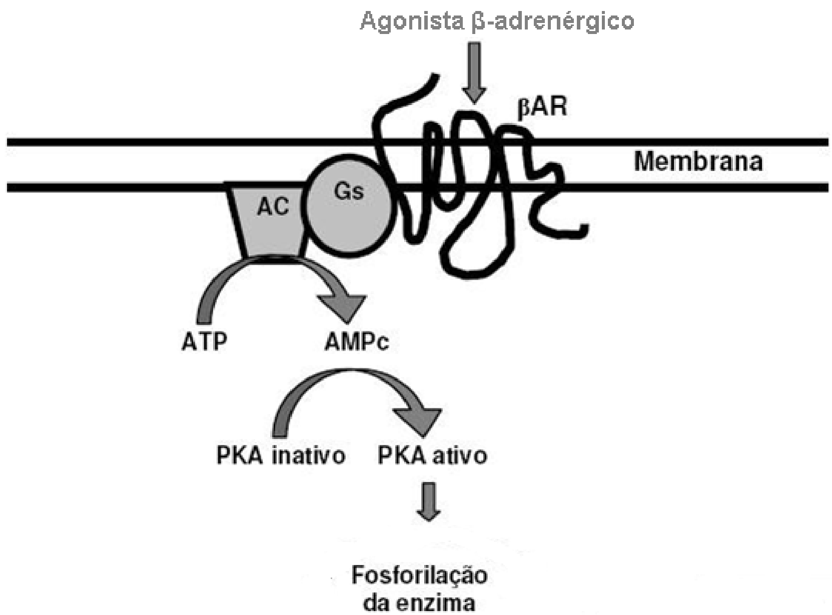

Figura 1 - Ação dos agonistas β-adrenérgicos na lipólise do tecido adiposo (MOODY et al., 2000).

Os efeitos dos agonistas β-adrenérgicos sobre a hipertrofia da célula muscular e aumento da massa magra são atribuídos à ativação dos β receptores. Alguns autores demonstraram que a utilização de substâncias que ativam os receptores β2, estimulam a síntese e inibem a degradação protéica (MOLONEY et al., 1991). Enquanto que, a ativação dos receptores β1 afeta somente a síntese protéica (BERGEN et al., 1989 citado por ZAGURY, 2002).

Segundo Aalhus et al. (1990), o aumento da massa muscular causado pela RAC é ocasionado pelo aumento no diâmetro das fibras musculares, ocorrendo principalmente nas fibras brancas e intermediárias.

É importante salientar que para a ractopamina alcançar o efeito esperado, deve-se disponibilizar maior quantidade de proteína bruta e aminoácidos (lisina), pois uma maior mobilização protéica é esperada quando estes aditivos são adicionados na dieta. A lisina tem sido considerada o primeiro aminoácido limitante na dieta de suínos à base de milho e farelo de soja. Isto ocorre, pois o metabolismo do animal exige maior quantidade desse aminoácido para aumentar a deposição de tecido magro (KESSLER, 1998).

Animais tratados com ractopamina devem consumir 30% a mais de lisina para atingirem resultados significativos de desempenho e qualidade de carcaça (MITCHELL et al., 1991; XIAO et al., 1999). Pereira et al. (2008) observaram que leitoas em terminação suplementadas com 0,87% de lisina digestível e 5 mg/kg de ractopamina apresentaram melhor desempenho produtivo e características de carcaça mais desejáveis, em comparação com o grupo alimentado com 0,67% de lisina.

2.3 EFICIÊNCIA DA RACTOPAMINA

A inclusão de ractopamina na dieta de suínos em fase de terminação tem demonstrado melhorar o desempenho zootécnico do animal, a eficiência alimentar, a menor deposição de gordura na carcaça e o aumento do rendimento de carne magra (STOLLER et al., 2003; SEE et al., 2004; WEBER et al., 2006; FERNÁNDEZ-DUEÑAS et al., 2008).

A resposta à ractopamina, e possivelmente a outros agonistas β-adrenérgicos, parece ser rápida. A medida em que o desempenho do animal alcança um pico, a eficiência torna-se platô e decresce ao longo do tempo, devido a desensibilização dos β receptores (DUNSHEA, 1993; WILLIAMS et al., 1994; MOODY et al., 2000; KELLY et al., 2003; SEE et al., 2004).

O sexo pode ocasionar diferenças no desempenho dos animais durante os períodos de crescimento e principalmente de terminação (UNRUH et al., 1996; LATORRE et al., 2004). Essas diferenças alteram o padrão de deposição do tecido magro e adiposo na carcaça e as propriedades tecnológicas da carne (UNRUH et al., 1996; ELLIS, 1998; LATORRE et al., 2004).

See et al. (2004) e Amaral et al. (2009) observaram diferenças em machos castrados e fêmeas alimentados com ractopamina. Machos foram mais pesados e cresceram mais rapidamente, por outro lado as fêmeas foram mais eficientes e depositaram maior quantidade de carne magra. Em contraste, pesquisa realizada por Poletto et al. (2009) obeservou maior espessura de toucinho em fêmeas alimentadas com RAC em comparação com machos castrados.

Amaral et al. (2009) constataram ainda que a suplementação com 5 mg/kg de ractopamina, durante 28 dias, é suficiente para melhorar o desempenho e a composição de carcaça de suínos. Além disso, a suplementação com 5 ou 10 mg/kg de ractopamina, mostrou-se economicamente viável.

3. MÉTODOS DE CASTRAÇÃO

3.1 CASTRAÇÃO CIRÚRGICA

O método de castração cirúrgica em suínos machos tem sido amplamente utilizado ao longo dos anos. Essa prática permite uma maior facilidade de manejo dos animais, pois estes se tornam mais dóceis. No entanto, a principal razão para a castração cirúrgica é a garantia de qualidade de uma carne livre de odores sexuais. Por outro lado, este método ocasiona profundas alterações no metabolismo animal, como: diminuição da eficiência alimentar, desenvolvimento muscular lento, menor retenção de nitrogênio e maior deposição proporcional de gordura e menor desenvolvimento de massa muscular, em comparação com machos inteiros, o que aumenta o custo de produção (BONNEAU, 1998; OLIVER et al. 2003). Além disso, a castração cirúrgica de leitões causa impacto na qualidade de vida e no seu rendimento como produto final, pois esta é realizada sem anestesia o que provoca dor e angústia, quantificados pelos aspectos de frequência cardíaca, atividade cerebral, vocalização e liberação de hormônios relacionados ao estresse (ZENG et al., 2002; HAYA et al., 2003). Podem causar também, aumento na mortalidade de animais (DUNSHEA et al., 2001) e inflamações crônicas ou infecções nesses leitões (POLEZE, 2007). Por todos esses fatores a rotina de castração cirúrgica na primeira semana de vida dos leitões tem sido apontada como um procedimento cruel e doloroso.

Em diversos países da Europa, como por exemplo a Espanha, a produção de machos inteiros jovens é bastante utilizada, onde é comum a obtenção de carcaças com peso inferior a 80 kg (WALSTRA et al., 1999; BAÑON et al., 2000). Dessa maneira, os animais não alcançam a maturidade sexual e a qualidade da carne é mantida (BAÑON et al., 2004).

A produção de suíno macho inteiro oferece diversas vantagens para o complexo agro industrial. Os machos inteiros, comparados com fêmeas e castrados, têm melhor eficiência alimentar, crescem mais rapidamente, produzem carcaças mais magras, diminuem a excreção de nitrogênio no meio ambiente, além de evitar o cruento método de castração cirúrgica (BONNEAU et al., 2000). Entretanto, o principal problema em produzir suínos machos inteiros está relacionado à qualidade da carne, a grande limitação desta prática está no aparecimento de odor sexual na carcaça. Por esse motivo, no Brasil, o abate generalizado de suínos machos inteiros é proibido pela legislação brasileira, conforme consta no artigo 121 do RIISPOA, Decreto 30.691 de 29.03.1952 e Circular 47 de 04.05.1988 alterado pelo Decreto 1255 de 25.06.1962 (BRASIL, 1952; BRASIL 1988).

3.1.1 Compostos Responsáveis Pelo Odor Sexual

O odor sexual ou odor de cachaço ocorre em alguns machos inteiros durante o período de maturidade sexual. Isto é causado principalmente pelo acúmulo de pelo menos um dos dois compostos, androstenona (5a-androst-16-ene-3-one; PATTERNSON, 1968) e escatol (3-methyl indole; VOLD, 1970). Este odor pode ser percebido durante a cocção, o que causa a rejeição da carne de animais não castrados pelo consumidor (BABOL et al., 2004).

Androstenona é um hormônio esteróide, produzido nos testículos, transportado via circulação sanguínea e secretado na saliva e no tecido adiposo (GOWER, 1972), reportado por causar forte odor de urina na carne. Escatol é produto da degradação do aminoácido triptofano por bactérias do intestino grosso e quando absorvido pelo trato gastrointestinal uma parte é transportada até o tecido adiposo, onde se acumula, e outra é eliminada através da urina. Este composto é relatado por causar intenso odor fecal na carne suína (BONNEAU et al., 1998; ABERGAARD e LAUE, 1993). Ambos compostos são lipofílicos e acumulam-se no tecido adiposo, causando odores desagradáveis e sabores estranhos, quando presentes em concentrações elevadas (BABOL, 1996).

3.2 IMUNOCASTRAÇÃO

A castração imunológica surge como uma possibilidade de se evitar o aparecimento do odor sexual, e ainda aproveitar os efeitos dos anabolizantes naturais, produzidos nos testículos dos machos inteiros, ao longo da sua vida produtiva (BONNEAU et al., 1982; SILVEIRA et al., 2006). Este método interfere nas características de comportamento do animal ao diminuir a agressividade e a atividade sexual; influencia fatores de produtividade ao melhorar a conversão alimentar, a velocidade de crescimento e aumentar a gordura intramuscular (JAROS et al., 2005).

A imunocastração é uma ferramenta que suprime temporariamente a produção de esteróides pelos testículos antes do abate. Funciona como uma vacina convencional, estimulando o sistema imunológico dos suínos a produzir anticorpos naturais contra o fator de liberação de gonadotrofinas, GnRF (JAROS et al., 2005; EVANS, 2006). O GnRF é um pequeno peptídeo (decapeptídeo) originário do hipotálamo com ação na glândula pituitária posterior para induzir a secreção do hormônio luteinizante (LH) e o hormônio folículo estimulante (FSH). Estes dois hormônios agem sobre as gônadas para estimular o desenvolvimento dos testículos e a produção de esteróides responsáveis pelas características de machos e crescimento muscular (ZENG et al., 2002; JAROS et al., 2005). Ao aplicar duas doses da vacina, oito e quatro semanas antes do abate, os anticorpos neutralizam o GnRF suíno e, consequentemente, bloqueiam a liberação de LH e FSH da glândula pituitária, a qual torna a bloquear a função testicular. Ao cessar o desenvolvimento dos testículos, estes são retidos altos no escroto e aparecem menos proeminentes. A produção de esteróides no testículo é suprimida e impede o metabolismo de substâncias envolvidas na ocorrência do odor (androstenona e escatol) na gordura (EVANS, 2006).

A razão pela qual os níveis de escatol são mais baixos em suínos castrados e fêmeas do que em suínos inteiros ainda não está bem elucidada. Entretanto, um estudo desenvolvido sobre o efeito do escatol e da androstenona na indução do citocromo P4502E1 pode oferecer uma explicação. O citocromo hepático P4502E1 (CYP2E1) parece estar envolvido no metabolismo do escatol (SQUIRE et al., 1997; BABOL et al., 1998). Foi observado que o escatol induz a expressão da proteína CYP2E1, enquanto a androstenona antagoniza este efeito. Deste modo, altas concentrações de androstenona podem previnir a indução do CYP2E1 pelo escatol. O efeito será uma redução no metabolismo do escatol e acúmulo no tecido adiposo (DORAN et al., 2002 citado por ALDAL et al., 2005).

Dunshea et al. (2001), Jaros et al. (2005), Silveira et al. (2006) e Zamaratskaia et al. (2008) relataram que a imunocastração (imunização contra o GnRF), utilizando a vacina Vivax® (Pfizer Animal Health, São Paulo, SP) foi eficiente em controlar os compostos responsáveis pelo odor sexual (androstenona e escatol) a níveis inferiores ao do limiar de detecção dos provadores, além de manter os níveis de testosterona comparáveis aos animais castrados cirurgicamente.

A imunocastração melhorou o desempenho zootécnico e contribuiu para aumentar a quantidade de carne (2,42 kg), diminuir a de gordura (0,77 kg) e acrescentar mais carne nos cortes de maior valor comercial, como o pernil, carré, a barriga e paleta. Isso representa uma vantagem econômica para a indústria da carne, pois assim atende os mercados de carne fresca e de produtos industrializados (cozidos e embutidos); (SILVEIRA et al., 2006).

Segundo Zamaratskaia et al. (2008), ao estudarem o comportamento de animais imunocastrados em comparação com animais castrados cirurgicamente, não encontraram diferenças significantes no tempo de repouso, locomoção e alimentação; animais imunocastrados também apresentaram reduzido comportamento sexual e agressivo

Segundo estudo realizado por Furnols et al. (2009), utilizando análise sensorial com provadores treinados, a carne suína de animais imunocastrados não apresentou diferença significativa para odor e sabor de androstenona e escatol quando comparada a de castrados cirúrgicos e fêmeas, enquanto que a carne proveniente de machos inteiros obteve maiores notas para ambos compostos.

4. RACTOPAMINA VS. BEM- ESTAR ANIMAL

O bem-estar é o estado de harmonia entre o animal e seu ambiente, caracterizado por condições físicas e fisiológicas apropriadas para garantir qualidade de vida ao animal (HURNIK, 1992).

O termo estresse foi usado pela primeira vez pelo médico endocrinologista Hans Selye, que definiu o estresse como sendo um fenômeno não específico que representa consequências no comportamento, na fisiologia e no estado emocional de um ser humano ou animal, em resposta a uma série de estímulos (SELYE, 1975; LUPIEN et al., 2007). O estresse pode ter uma função positivo, pois animais sob estresse têm sua homeostasia ameaçada e em resposta a isso, desenvolvem mecanismos de adaptação (MOBERG, 2000). Segundo Terlouw (2005) estresse agudo ou crônico pode ser considerado como o estado em que o animal é impossibilitado de expressar seu comportamento natural (impossibilidade de deitar, problemas relacionados à fome, sede, dor ou doenças), ou do seu estado mental (isolamento social, medo e frustração). Sendo o estresse um dos principais indicadores utilizado para avaliar o bem-estar animal, é preciso ficar atento e minimizar os estímulos estressantes a que os animais de produção são submetidos ao longo de sua vida.

Os efeitos positivos da adição da ractopamina em suínos na fase de terminação, no que diz respeito ao desempenho zootécnico do animal, estão bem estabelecidos (RICKS et al., 1984; MOODY et al., 2000; GONZALES et al., 2010). No entanto é preciso investigar possíveis efeitos desse composto sobre o comportamento e a fisiologia animal.

Marchant-Forde et al. (2003) observaram que animais suplementados com ractopamina durante quatro semanas, apresentaram elevação dos batimentos cardíacos, maiores níveis de catecolaminas (epinefrina e norepinefrina), e por consequência, tornaram-se mais ativos, alertas e demoram mais tempo para se acalmar após uma situação estressante, além de geralmente, serem mais difíceis de manejar. A ractopamina foi, também, associada ao aumento da agressividade principalmente em fêmeas (POLETTO et al., 2010ab).

Em contrapartida, um estudo realizado por Athayde (2010) avaliou o bem-estar de suínos em condições comerciais brasileiras e observou que a suplementação de ractopamina não alterou o comportamento, a incidência de lesões de pele e nem a concentração de cortisol e lactato. Somente os níveis sanguíneos de creatina fosfoquinase foram mais elevados em suínos que consumiram a dieta contendo ractopamina.

O bem-estar animal pode ser avaliado indiretamente através de observações comportamentais e vocalizações, em resposta a agentes estressantes, além de medidas fisiológicas, como a concentração sanguínea do hormônio cortisol, atividade enzimática da creatina fosfoquinase e lactato desidrogenase (GRANDIN, 1998; FÀBREGA et al., 2004; TERLOUW et al., 2005; SUTHERLAND et al., 2008). Em animais de produção, informações adicionais do estresse *ante mortem* podem ser obtidas por avaliações da carcaça logo após o abate (GUÀRDIA et al., 2009).

4.1 FORMAS DE AVALIAÇÃO DO ESTRESSE

4.1.1 Vocalização

A vocalização é conhecida por ser um importante meio de comunicação em suínos (KILEY, 1972). Por exemplo, porcas emitem grunhidos durante a amamentação de uma forma relacionada com o tempo de disponibilidade de leite (FRASER, 1974), leitões vocalizam quando se encontram isolados (WEARY e FRASER, 1995; WEARY et al., 1997; COLONNELLO et al., 2010), famintos (APPLEBY et al., 1999), com frio (HILMAN et al., 2004), com dor (LEIDIG et al., 2009), lesionados ou quando manipulados por seres humanos (WEARY et al 1998; MARCHANT et al., 2001). Pesquisa realizada por Grandin (1998) indicou que 99% das vocalizações de bovinos durante o manejo e insensibilização em um abatedouro comercial foram associados com eventos agressivos, como utilização de bastão elétrico, tentativas de atordoamento fracassadas, escorregão no *box* de atordoamento ou excesso de pressão a partir do *restrainer*. Diversos autores constataram que vocalizações com baixas frequências (grunhidos) são utilizadas no contato social, enquanto que as vocalizações de altas frequências (gritos) estão relacionadas com a reação dos suínos a situações estressantes (KILEY, 1972; FRASER, 1974; WEARY et al., 1997;. TAYLOR e WEARY, 2001; MANTEUFFEL et al., 2004; DÜPJAN et al., 2008).

Pesquisa realizada por Hillmann et al. (2004) constatou que vocalizações de alta freqüência foram importantes indicadores de estresse térmico em suínos submetidos a baixas temperaturas. Considerando que o som emitido pelos animais é resultado de um estado emocional particular, a análise da vocalização dos animais de produção tem se tornado um interessante indicador de estresse. Uma ferramenta não-invasiva cada vez mais importante para avaliar o bem-estar animal (WEARY e FRASER, 1995; SCHRADER e TODT, 1998; WEARY et al., 1998; MANTEUFFEL et al., 2004; SCHÖN et al., 2004; PUPPE et al., 2005).

A frequência é uma das grandezas mais relevantes na caracterização do som e traduz o número de ciclos por unidade de tempo numa forma de onda periódica. Em geral, as altas frequências são características de sons agudos e as baixas freqüências de sons graves. A unidade de medida universal da frequência é o Hertz (Hz), que traduz o número de ciclos por segundo (SANTOS, 1991).

A primeira frequência representada pela mais baixa e forte frequência de um som é a frequência fundamental. Variações da frequência fundamental podem estar relacionadas a variações de tensão, comprimento e massa das pregas vocais. Outras formantes surgem consecutivamente à frequência fundamental e participam da composição da onda sonora. Este conjunto de sons secundários, também conhecidos como sons harmônicos que acompanham o som principal (frequência fundamental) formam o timbre, que é a qualidade capaz de diferenciar dois sons de mesma frequência e intensidade (SANTOS, 1991). A intensidade do som está associada a quantidade de energia contida em um movimento vibratório, o grau de força da vocalização (YEON et al., 2006). A intensidade de um som pode ser medida através de dois parâmetros: a densidade de potência transmitida no movimento vibratório (W/m^2 ou W/cm^2) ou a pressão do ar causada pela onda sonora (N/m^2, Pascal (Pa) ou BAR); (SANTOS, 1991).

A amplitude está relacionada a intensidade com que percebemos o som. A variação da amplitude é proporcional à variação da pressão atmosférica causada pela onda sonora. A medida da amplitude é representada em unidade de pressão e geralmente é convertida a uma escala de decibel (dB) (SANTOS, 1991).

4.1.2 Parâmetros fisiológicos do estresse

4.1.2.1 Cortisol

Uma série de sistemas fisiológicos são ativados em resposta ao estresse. Uma resposta comum ao estresse é a ativação do eixo hipotálamo-hipófise adrenal. Isso envolve a liberação sequencial do hormônio liberador de corticotropina (CRH) e a vasopressina do hipotálamo, seguido do hormônio adrenocorticotrófico (ACTH) liberado pela hipófise anterior que controla a liberação periférica de glicocorticóides, como o cortisol, a partir do córtex adrenal. O cortisol é reconhecido como um importante indicador de estresse, pois os animais elevam sua concentração em resposta ao estresse fisico e/ou psicológico sofrido, que estimula a gliconeogênese e lipólise mobilizando os estoques de energia para preparar seu organismo com suprimento extra de energia, permitindo a “reação de luta ou fuga” (BROOM e FRASER, 2007). A quantificação desse hormônio no plasma ou no soro sanguíneo tem sido amplamente utilizada para avaliar os aspectos fisiológicos de bem-estar animal (GRANDIN, 1997; TURNER et al., 2002; MARIA et al., 2004; COPPOLA et al., 2006; CHAI et al., 2010). Os níveis de estresse do animais dependem da situação a qual o animal encontra-se, e da avaliação do animal da situação. Cada indivíduo é único, dependendo da sua origem genética e sua experiência anterior. Portanto, sua avaliação da situação, e o resultado de seu nível de estresse são subjetivos, ou seja, indivíduo dependente (TERLOUW, 2005). Portanto, para avaliar as condições de estresse a que um animal é submetido deve-se sempre comparar as concentrações de cortisol entre dois tratamentos, assim o que produzir o menor valor médio de cortisol poderá ser considerado como menos estressado (GRANDIN, 1997).

Diversas situações de estresse podem levar a um aumento nas concentrações de cortisol sanguíneo como: embarque e desembarque do caminhão (AVEROS et al., 2007), alta densidade (CHAI et al., 2010), mistura de lotes nas baias de espera, exposição a condições extremas de temperatura (BECKER et al., 1997), subida e descida de rampas (LEWIS et al., 2008), manejo pré abate e insensibilização (SANTANA, et al., 2009).

4.1.2.2 Creatina fosfoquinase e Lactato desidrogenase

As enzimas creatina fosfoquinase (CPK) e lactato desidrogenase (LDH) estão envolvidas no metabolismo da glicólise muscular, a energia imediata para a contração muscular é derivada do ATP, formando ADP + Pi. A quantidade de ATP na fibra muscular é limitada e a refosforilação do ADP deve ocorrer para que a contração possa continuar. A CPK é responsável por catalisar a desfosforilação da creatina fosfato para produção de ATP. Após a quebra da glicose e formação de piruvatos, a enzima LDH catalisa a conversão do piruvato em lactato (ABERLE et al., 2002). Situações de estresse intenso podem levar à exaustão e intensa degradação do glicogênio muscular, provocando a liberação dessas enzimas citoplasmáticas das fibras musculares para a circulação sanguínea (FÀBREGA et al., 2004; SUTHERLAND et al., 2008).

Creatina fosfoquinase (CPK) é um indicador altamente sensível e específico de lesão muscular em animais domésticos. Embora a CPK seja encontrada tanto no músculo cardíaco quanto no esquelético, elevações desta enzima estão mais comumente associadas à lesões musculares. O LDH também está presente em diversos órgãos, porém o aumento da sua concentração extracelular tem sido utilizado para detecção de lesão muscular, comum indicador de estresse físico (CHAI et al., 2010). Averos et al. (2007) observaram significativo aumento dos níveis sanguíneos de CPK e LDH em suínos, após o desembarque e sangria. Chai et al. (2010) avaliaram as concentrações de LDH e CPK em diferentes densidades e tempo de transporte e observaram o aumento da concentração dessas enzimas em suínos submetidos a alta densidade, e maiores níveis de LDH para animais transportados por períodos mais longos. Os efeitos nos níveis de CPK e LDH podem também ser utilizados para outras avaliações de estresse, como avaliação do efeito do gene halotano heterozigoto (Nn) e homozigoto negativo (NN) frente a situações estressantes, como observado por Fábrega et al. (2002).

4.1.3 Escoriação da carcaça

A presença de escoriações na carcaça de suínos é um reflexo de más condições de bem-estar animal, além de ser considerada um grande problema comercial por afetar a qualidade de carne. Diversos fatores podem causar escoriações na pele dos animais, tais como o manejo de embarque e desembarque (NANNI COSTA et al., 1999; GUÀRDIA et al., 2009), tipo de piso do caminhão, densidade (BARTON-GADE e CHRISTENSEN, 1998; GUÀRDIA et al., 2009), condução de grandes grupos de animais que levam a resistência e brigas (DRIESSEN e GEERS, 2000), tempo de transporte, repouso nas baias de espera (FRANQUEZA et al., 1998; NANNI COSTA et al., 2002; GUÀRDIA et al., 2009) e tempo de jejum (FAUCITANO et al., 1998). Características individuais, como agressividade e susceptibilidade ao estresse, têm importante papel na incidência de danos na pele (BOLHUIS et al., 2005). A agressividade está relacionada ao sexo e genética dos animais. Alguns autores observaram que os machos inteiros (MOSS, 1978; GUISE e PENNY, 1989; BROWN et al, 1999) são mais agressivos devido a seu comportamento sexual e tiveram a maior ocorrência de escoriações na pele. Apesar de pesquisa realizada por Poletto et al. (2010) ter observado aumento na agressividade de fêmeas em comparação com machos castrados, Guàrdia et al. (2009) não encontraram nenhuma evidência de efeito de sexo, entre machos castrados e fêmeas, na incidência de escoriações. Suínos podem responder de forma diferente ao estresse de acordo com suas características individuais, entretanto de uma forma geral, a mistura de lotes na pocilga aumenta a agressividade devido ao estabelecimento de uma nova hierarquia e, consequentemente, aumenta a incidência de escoriações na carcaça (WARRISS, 1996; GEVERINK et al., 1998; TURNER et al., 2006; GUÀRDIA et al., 2009). Índices de escoriações da pele geralmente estão mais associados às condições de estresse psicológico e desgaste físico que ao estresse de curto prazo (WARRISS et al., 1998). Poletto et al. (2010) observaram que fêmeas alimentadas com RAC apresentaram comportamento mais agressivo. Uma vez que a ractopamina tem sido investigada, quanto ao aumento da agressividade em suínos, escoriações de pele são um bom indicador para medir esse parâmetro.

4.1.4 Rigor mortis

A avaliação do desenvolvimento precoce do *rigor mortis* pode fornecer informações sobre o metabolismo *post mortem*, o qual pode ser um indicativo da extensão de estresse a qual o animal foi submetido minutos ou horas antes do abate (BARTON-GADE et al., 1996). Animais mais susceptíveis ao estresse têm seu metabolismo acelerado e podem apresentar instalação precoce do *rigor mortis* devido à redução do estoque de substrato presente no músculo (WARRISS et al., 1998). Além disso, o desenvolvimento precoce de *rigor mortis* está correlacionado com a concentração sanguínea de cortisol, a qual aumenta em resposta ao estresse (WARRISS et al., 2003).

4.1.5 Lesões de Casco

A predisposição para lesões de casco em suínos pode ter distintas origens, como o sobrepeso dos animais, deficiência nutricional (biotina), manejo de limpeza das baias e condições de piso (LOPEZ et al., 1997). Animais mais ativos e com maior predisposição à agressividade favorecem o aparecimento de lesões de casco, devido à maior movimentação dentro da baia. Poletto et al. (2009) observaram maior incidência de lesões nos cascos traseiros de suínos dominantes quando comparados com animais considerados submissos. Este resultado foi associado a uma maior expressão de comportamento agonista em animais dominantes, incluindo brigas para manter sua posição hierárquica na baia. Poletto et al. (2009) também relataram que suínos alimentados com RAC apresentaram aumento em lesões de casco em relação ao grupo controle. Penny et al. (1994) observaram que suínos alimentados com um composto da mesma categoria que a RAC, salbutamol β-adrenérgico, obtiveram aumento na frequência e gravidade das lesões de casco em suínos em terminação.

5. RACTOPAMINA VS. QUALIDADE DA CARNE

A maioria dos estudos relacionados à ractopamina é direcionada ao seu efeito no desempenho zootécnico do animal e na sua capacidade de aumentar a síntese protéica e diminuir a deposição de gordura na carcaça suína. Entretanto, maior atenção deveria ser direcionada às possíveis alterações que este composto pode causar na qualidade da carne suína, pois diversos autores têm demonstrado divergência nos resultados encontrados em suas pesquisas.

5.1 PARÂMETROS FÍSICO-QUÍMICOS

5.1.1 pH

O pH é uma característica altamente relacionada com a qualidade da carne, uma vez que influencia a cor, a capacidade de retenção de água e a textura (ZAGURY, 2002). O metabolismo do glicogênio tem importante papel na expressão do pH. Um dos principais problemas que acomete a carne suína é a carne *pale, soft e exudative* (PSE). Sabe-se que dentro das principais causas que induzem à incidência de carne PSE, pode-se mencionar o estresse que sofre o animal no momento do abate, a genética (portadores do gene halotano), assim como as condições de refrigeração na fase *post mortem* das carnes (SIERRA et al., 2005). Importantes aspectos do uso de agonistas β-adrenérgicos na ração de suínos foram relatados por Warriss (1989). Foi observado que animais suplementados com este composto, através do mimetismo dos efeitos naturais das catecolaminas, poderiam desenvolver a carne PSE ou, ainda, poderia haver estímulo da glicólise e assim promover o consumo de glicogênio muscular *ante mortem*, resultando em menor produção e acúmulo de ácido láctico na carcaça após o abate. Entretanto, estudos mais recentes com ractopamina não confirmaram essas hipóteses, uma vez que o pH_{24h} (STOLLER et al., 2003; WEBER et al., 2006; XIONG et al., 2006; STHAL et al., 2007) e o pH_{1h} (CARR et al., 2005; WEBER et al., 2006; XIONG et al., 2006) das carcaças não foi alterado quando da adição dessa substância. Bridi et al. (2006), ao analisarem o efeito da adição de ractopamina na dieta de suínos do genótipo halotano heterozigoto e homozigoto, não observaram aumento da incidência de carne PSE.

5.1.2 Cor

A cor é considerada a mais importante característica sensorial da aparência da carne fresca, pois este atributo é determinante para a decisão de compra do consumidor.

Na cor objetiva, determinada pelo método CIELAB, a luminosidade (L*) é a medida relacionada à qualidade de carne suína, pois o L* é um dos fatores que classifica uma carne como PSE ou DFD.

A luminosidade da carne suína parece não ser influenciada pela adição de ractopamina na dieta (ARMSTRONG et al., 2004; CARR et al., 2005; STHAL et al., 2007). O componente a * da cor é uma medida do teor de vermelho e pode ser usado como uma indicação da quantidade de oximioglobina presente durante o período de *blooming* da carne (JOHANSSON, 1989). Portanto, a diminuição nos valores de a * sugerem redução na quantidade de oximioglobina na carne produzida por suínos alimentados com RAC (UTTARO et al., 1993; AALHUS et al., 1990). Estudos mais recentes também observaram que a ractopamina tem diminuído os valores de a* (CARR et al., 2005

FERNANDEZ-DUEÑAS et al. 2008; APPLE et al., 2008) e b* (CARR et al., 2005; STHAL et al., 2007; APPLE et al., 2008). Diversos autores não encontraram nenhum impacto da ractopamina nos valores de a* (ARMSTRONG et al., 2004; FERNANDEZ-DUEÑAS et al., 2008; GONZALES et al., 2010).

5.1.3 Perdas por exsudação e cocção

A perda de peso por exsudação está relacionada com a capacidade da carne crua em reter água no seu interior. É uma propriedade de importância fundamental em termos de qualidade, pois muitas propriedades físicas da carne (cor, maciez e suculência) são parcialmente dependentes da capacidade de retenção de água. A formação de ácido lático e a consequente queda do pH *post mortem,* próximo ao ponto isoelétrico das proteínas miofibrilares, é responsável por uma redução nos grupos reativos das proteínas disponíveis para ligação com a água; como resultado ocorre maior perda de peso por exsudação da carne (ABERLE et al., 2002). Os mesmos fatores que agem sobre a perda de peso por exsudação, também influenciam a perda de peso da carne cozida, uma vez que as diferenças relativas se mantêm após o aquecimento. Entretanto, o método de cocção, a temperatura e tempo podem aumentar as perdas da carne cozida, pois temperaturas elevadas destroem a estrutura da carne e fundem suas gorduras (PARDI et al., 2006).

Diversos estudos observaram que a porcentagem de perda por exsudação não é comumente afetada pela ractopamina (UTTARO, et al., 1993; STOLLER et al., 2003;. WEBER et al., 2006; APPLE et al., 2008). No entanto, Carr et al. (2005) relataram que, quando uma concentração elevada de ractopamina (20 mg/kg) foi utilizada, observou-se redução na perda por exsudação no M. *longissimus dorsi.* O mesmo ocorre com a perda de peso por cocção, onde diversos autores também não observaram diferenças significativas em suínos suplementados com ractopamina

(STOLLER et al., 2003; STHAL et al., 2007; APPLE et al., 2008; FERNÁNDEZ-DUEÑAS et al., 2008) e somente quando 20 mg/kg de ractopamina foram adicionados à dieta, houve diminuição da perda de peso por cocção (UTTARO et al., 1993).

5.1.4 Força de cisalhamento

O aumento da síntese proteíca e a dimuição da deposição de tecido adiposo em suínos alimentados com ractopamina resultou em certa preocupação com a qualidade da carne, em especial com a maciez.

Diversos estudos reportaram uma relação inversa entre dieta contendo ractopamina e maciez da carne (UTTARO et al., 1993; AALHUS et al., 1998; XIONG et al., 2006; STAHL et al., 2007; FERNANDEZ-DUEÑAS et al., 2008). Fernández-Dueñas et al. (2008) também relataram maiores valores de força de cisalhamento em carne de suínos alimentados com ractopamina, entretanto provadores treinados não conseguiram detectar essa diferença. Apple et al. (2008), Merkel et al. (1990) e Stites et al. (1991) não encontraram diferenças significativas de textura entre lombos do tratamento com ractopamina (10 mg/kg) e do controle.

Xiong et al. (2006) atribuiram a diminuição da maciez de carne de suínos suplementados com ractopamina ao aumento da atividade de calpastatina e diminuição na atividade de calpaína no músculo. Estes resultados também demonstraram que o tempo de maturação da carne deve ser levado em conta, uma vez que o efeito da ractopamina sobre o aumento dos valores de força de cisalhamento diminuiu ao longo do tempo e ficaram iguais ao grupo controle no décimo dia de maturação. A diminuição da maciez em carnes provenientes de suínos alimentados com ractopamina pode também ser causada pela maior quantidade de ligações de colágeno do que o normal (DAWSON et al., 1990 citado por LAWRIE e LEDWARD, 2006). Além disso, Aalhus et al. (1992) sugeriram que a ractopamina aumenta o diâmetro da fibra, o que pode estar associado com a diminuição da maciez independente do tecido conectivo ou da idade do animal.

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ARTIGO 1: THE IMPACT OF RACTOPAMINE HYDROCHLORIDE ON WELFARE OF SLAUGHTER PIGS: VOCALIZATION, CARCASS MEASUREMENTS, HOOF LESIONS, AND BLOOD PARAMETERS OF STRESS

O artigo a seguir está redigido de acordo com as normas para publicação na revista *Journal of Animal Science.*

The impact of ractopamine hydrochloride on welfare of slaughter pigs: vocalization, skin damage, *rigor mortis*, hoof lesions, and blood parameters of stress

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ABSTRACT:

The addition of ractopamine (RAC) on the diet of finishing pigs has been widely used to improve production performance and it also has become a concern in the fields of animal welfare. The objective of this study was to investigate the effects of RAC feeding on animal vocalization, blood parameters of stress, hoof lesions, skin damage and *rigor mortis*. A total of 310 gilts, immunocastrates and barrows from two different farms and distinct genetic crosses [Tempo sires and Topigs 40 dams from farm A (n=202) and AGPIC 337 sires and CB 22 dams from farm B (n = 108)] were randomly assigned to receive the RAC treatment (7.5 mg/kg), 3 weeks before slaughter, or the conventional diet without RAC. Pigs from each farm were slaughtered at different commercial slaughterhouses and chilled for 24 h at 4° C. Only in farm A vocalizations were recorded for each treatment within sex condition, during the animals' conduction to the stunning box. Blood samples were collected at exsanguination to determine the enzymatic activity of creatine phospho-kinase (CPK), lactate dehydrogenase (LDH) and the levels of cortisol. Front hooves were accessed, post-slaughter, for 3 distinct lesions categories: splits, crack-erosions and bruises. After scalding and evisceration carcasses were evaluated for early rigor development and skin damage score. Ractopamine treatment increased the amplitude ($P < 0.01$) and the sound intensity ($P = 0.01$) of pigs' vocalization. However, RAC-fed pigs had no important impact ($P \geq 0.09$) on blood parameters of stress, hoof lesions, skin damage and *rigor mortis* development. In general, gilts presented lower ($P < 0.01$) parameters of stress than immunocastrates or barrows, with the exception for measurements of cortisol levels, where RAC fed gilts had the highest ($P < 0.03$), concentration. Immunocastrated pigs had some parameters of stress greater than barrows ($P \leq 0.02$), such as the incidence of bruises within animals from farm A, total and shoulder skin damage. On the other hand, for farm B barrows also more hoof lesions than immunocastrates, such as crack-erosions ($P < 0.01$) and the incidence of splits ($P < 0.03$). The farms and processing plants influenced the incidence of some welfare parameters. Animals from farm A presented greater parameters of stress ($P < 0.05$), including splits, bruises, development of rigor mortis and especially LDH blood levels, than farm B. RAC feeding affected cortisol in gilts and some vocalization parameters in gilts and immnocastrates. For sex category was difficult to point with category was more susceptible to stress, once different results were observed for each sex in different stress parameters measured. However, is important that future studies consider the pigs'sex when evaluating RAC effects, once this experiment showed that it may affect RAC treatments responses.

Key words: blood parameters of stress, immunocastration, pigs' welfare, ractopamine, vocalization.

INTRODUCTION

The inclusion of ractopamine hydrochloride (**RAC**) in finishing pigs diets has been shown to improve animal performance. As a repartitioning agent, that redirects nutrients away from fat deposition and toward lean tissue deposition (Ricks et al., 1984; Moody et al., 2000; Gonzales et al., 2010). The use of RAC has been proved to improve growth rate, feed-conversion rate (Williams et al., 1994; Stoller et al., 2003), average daily gain (Jones et al., 2000; See et al., 2004) and carcass leanness (Weber et al., 2006). RAC is extensively used for growth enhancement in late finishing pigs in the United States (Fernández-Dueñas et al., 2008) and has been also widely used by Brazilian swine producers. Although the positive effects of RAC feeding on production performance are well established, limited researches are available about its effects on animal welfare. Marchant-Forde et al. (2003) reported that pigs fed RAC were more difficult to handle and had elevated heart rates and catecholamine concentrations after 4 weeks of administration. RAC feeding was also associated with greater aggressiveness in gilts and dominant barrows (Poletto et al., 2010) and more front and rear hoof lesions in finishing pigs at slaughter (Poletto et al., 2009).

Thus, our hypothesis is that RAC feeding could increase the stress parameters in finishing pigs. The aim of the present study was to investigate subjectively and objectively some parameters of stress to determine the possible impact of RAC feeding and sex on pigs' welfare in two different commercial farms.

MATERIALS AND METHODS

Animals

This study involved 310 pigs (95 gilts, 107 immunocastrates and 108 barrows), weighing on average of 108–129 kg. Animals were born and housed in two different commercial farms representing distinct genetic crosses: Tempo sires × Topigs 40 dams from farm A (n=202) and AGPIC 337 sires × CB 22 dams from farm B (n = 108). The pigs designated to be immunocastrated received two doses of vaccine (Vivax®, Pfizer Animal Health, São Paulo, SP), 8 and 4 weeks before slaughter, and the barrows were physically castrated in the first week of their lives. For both farms pigs were provided with ad libitum access to a single spaced feeder and 2 nipple drinkers at all times. The animals were housed in a naturally ventilated finishing barn on solid concrete floor with a space allowance of 1.0 m^2/pig. At the finishing stage 7.5 mg/kg of ractopamine hydrochloride (RAC; Ractosui®, Ourofino Agronegócio, Cravinhos, SP) was incorporated into the conventional diet, based on corn and soybean, of half the animals during 21 days (± 2 days) before slaughter. The other half was used as a control group, receiving the conventional diet without RAC. At the end of finishing period, pigs from farm B were transported on a commercial truck for approximately 10 km, during 30 minutes, to a commercial pork processing plant (Frigorífico Bressiani, Capivari, SP) and two weeks later pigs from farm A were also transported on a similar commercial truck, but for approximately 250 km during four hours to another commercial pork processing plant (Frigorífico Mondelli, Bauru, SP). During transport, unfamiliar animals were mixed, but at the lairage pigs were penned with the same group from commercial farm, with a stocking density of approximately 2.0 m^2/pig, in pens of 15–20 individuals, with drinking water available throughout the lairage period. After an 8-h resting (Plant A) and 12-h resting (Plant B) period at the plant, animals were slaughtered by an electrical stunning (350–450V, 1–2A). The average carcass weight of the pigs was 96.22 kg. Humane slaughter was conduct in accordance with the Sanitary and Industrial Inspection Regulation for Products of Animal Origin (Brasil, 1997).

Vocalization Measurements and Analysis

This evaluation was conducted only in animals from farm A. For each treatment, 10 samples of pigs´vocalization were recorded in the way to the stunning box. Each sample was collected of a group of approximately three pigs during one minute each. For capturing the acoustic signal was used a unidirectional microphone, Yoga, held approximately 20 cm from the mouth of the pigs. The microphone was connected to a digital recorder, Marantz PMD 660, where the signals were recorder at a frequency of up to 44,100 Hz. The vocalization data collected were analyzed by Praat® software and the background sounds were excluded. For each signal it was analyzed sound intensity (dB), amplitude (dB), frequency (Hz) and fundamental frequency (Hz), Table 1.

Table 1. Variables measured for vocalization analysis

Measurment	Unit	Description	Reference
Sound intensity	dB	The degree of strength of vocalization	Yeon et al., 2006
Fundamental frequency	Hz	The lowest frequency band for which there is substantial energy is called the first format. This is the first harmonic of resonanse	Yeon et al., 2006
Amplitude	dB	Highest amplitude in the mean spectra of the call	Marx et al., 2003
Frequency	Hz	Mean values of the first four formants[a], harmonics, of resonance	Yeon et al., 2006

[a]Each formant is the convergence degree of sound energy in special frequency. It follows that only a limited number of frequencies will be present in the vocal output at any one time. These resonances are called formants. Formants can represent the characteristics of vocalization (Yeon et al., 2006).

Physiological measurements

Blood samples of an avarege of ten pigs were randomly taken at exsanguinations, from each sex condition with and without RAC in both farms, for determination of blood parameters of stress such as cortisol, creatine phospho-kinase (CPK) and lactate dehydrogenase (LDH). Blood samples were collected in plastic cups and quickly separated into three tubes (10 ml). The tubes were left to stand until blood coagulation and subsequently centrifuged using a portable centrifuge model Excelsa Baby II, Fanem at 3.500 rpm/8 min at room temperature to obtain serum. Blood serum was stored in liquid nitrogen at -196° C for subsequent analysis of enzymatic activity of CPK, LDH and hormone concentration of cortisol. Analyses of serum activities of CPK and LDH were performed by spectrophotometry using commercial kits purchased from Bioclin, Quibasa Química Básica Ltda, Belo Horizonte, MG. The concentration of cortisol was determined by the ADVIA Centaur Cortisol assay (Bayer Health Care, São Paulo, SP) which is a competitive immunoassay using direct chemiluminescent technology.

Hoof Lesions

After slaughter, pigs were hanging for vertical exsanguination and all individual animals (n=310), from both farms, had their front hooves evaluated for presence of lesions. Lesions were classified as 3 distinct categories: splits (Figure 1d; apparent as separation of the hoof wall away from the underlying layer), cracks-erosions (Figure 1c; false sand cracks in the hoof wall and erosions in the solar layer), and bruises (Figure 1b; distinct areas of dark discoloration in the sole and behind the hoof wall) as described by Polleto et al. (2009). The presence or absence of these lesions was counted in individual animals and the incidence of each lesion per treatment was expressed as percentage.

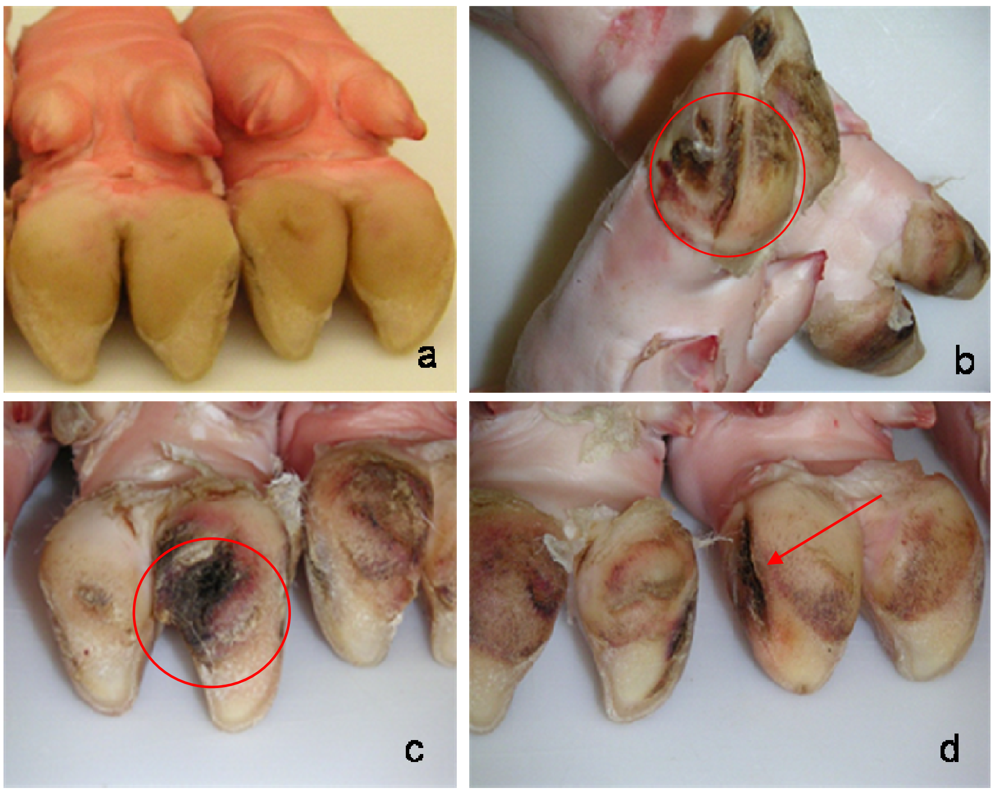


Figure 1. Hoof lesions evaluated: a) normal hooves, b) bruises, c) cracks-erosions, and d) splits. Source: Rosangela Poletto

Skin damage and rigor mortis

After scalding and evisceration, carcasses were assessed for quality as described by Barton Gade et al. (1996). Slaughter pigs from both farms (n = 310) were subjectively evaluated for rigor development (presence or absence) and skin damage (4 point scale; 1 = no skin damage, 2 = slight skin damage, 3 = skin damage affecting quality, 4 = extreme skin damage with possible rejection of tissue). The extent of skin damage was evaluated in the rear, middle and shoulder area of each half carcass (Figure 2).

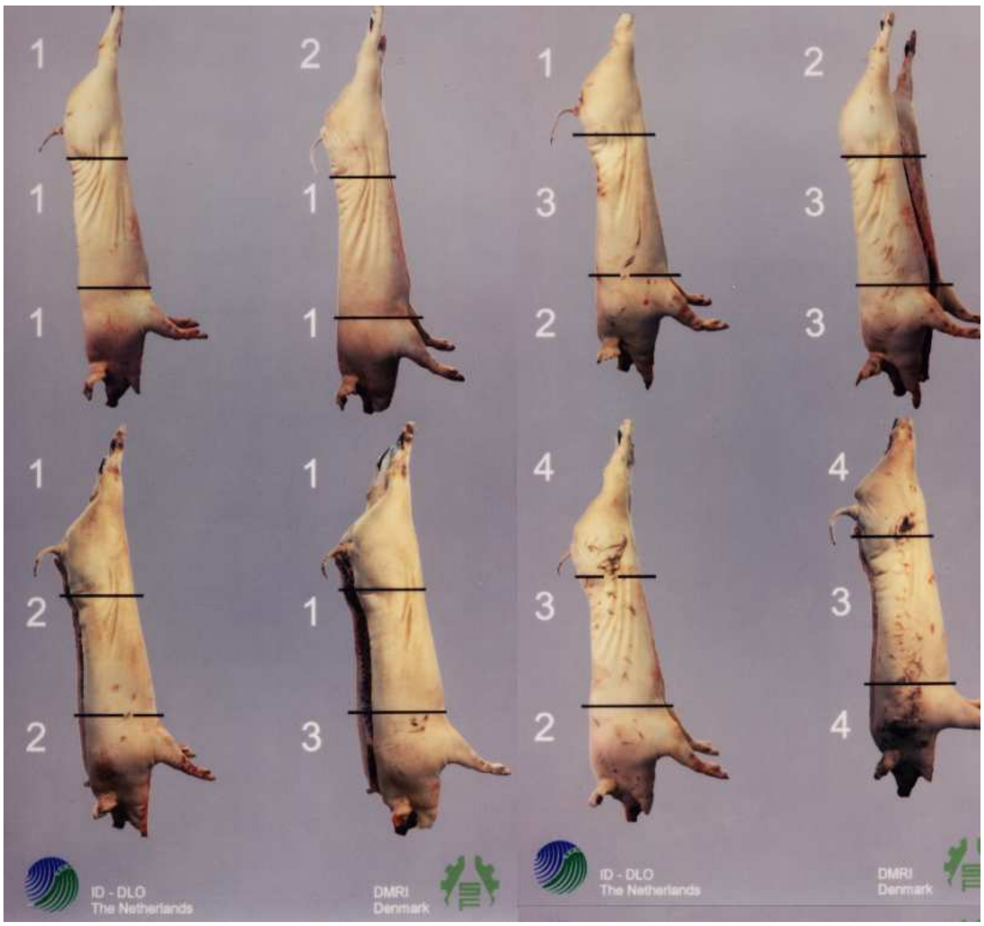


Figure 2. Photographic scale used to assess skin damage.

Statistical Analysis

Physiological measurements (CPK, LDH and cortisol) and skin damage score were analyzed as a factorial arrangement of 2 x 2 x 3 with the main effects of farm (Farm A vs. Farm B) RAC level (0 vs. 7.5 mg/kg) and sex condition (immunocastrates vs. barrows vs. gilts). Vocalization was analyzed as a factorial arrangement of 2 x 3, once this measurment was only taken in animals from farm A. An ANOVA was generated using the GLM procedure (SAS Inst. Inc., Cary, NC). Assumptions of ANOVA were checked by the family of optimal transformation of Box-Cox (1964) and the test of Hartley (1950) to verify the homogeneity of variance. The experimental unit for the ANOVA was the animal, and fixed (main) effects included in the model were RAC, sex condition, and farm, as well as the 2- and 3-way interactions. Least squares means were computed for main and interactive effects and were separated statistically using *F*-test (PDIFF). Tukey test ($p < 0.05$) was applied for the variables that showed difference. Hoof lesions was analyzed as a factorial arrangement of 2 x 2 x 3 and rigor mortis as a factorial arrangement of 2 x 3, once animal from farm B were not incorporated in the model because the early development of rigor mortis was not find in any of the carcasses evaluated. The model used was Binomial Logit link function ($P < 0.05$) for both variables. Data were generated using the GLIMMIX procedure (SAS Inst. Inc.). Statistical differences were declared at $P < 0.05$ level.

RESULTS

The *P*-values of main effects, as well as the 2- and 3-way interactions for all measurements studied are presented in Table 2.

Table 2. P-values and coefficient of variation (CV) for main effects of farm (F), sex (S), ractopamine (R) and interactions

	P-value (Main effects)			*P-value* (Interactions)				CV (%)
	Farm	Sex	Ractopamine	F x S	F x R	S x R	F x S x R	
Sound intensity (dB)	-	0.74	0.02	-	-	0.03	-	17.5
Amplitude (dB)	-	0.81	<.0001	-	-	0.01	-	51.2
Fundamental frequency (Hz)	-	0.94	0.95	-	-	0.15	-	11.2
Frequency (Hz)	-	0.21	0.53	-	-	0.30	-	3.0
Cortisol (ng/ml)	0.09	0.39	0.21	0.23	0.39	0.05	0.97	25.6
CPK (UI/L)	0.21	0.19	0.20	0.99	0.43	0.41	0.06	53.7
LDH (UI/L)	<.0001	0.37	0.89	0.03	0.54	0.14	0.77	26.6
Split (%)	0.05	0.57	0.11	0.03	0.002	0.90	0.56	-
Crack-erosion (%)	<.0001	0.01	0.42	0.18	1.00	0.39	0.29	-
Bruise (%)	0.03	0.02	0.09	0.02	0.34	0.06	0.003	-
Shoulder	0.18	0.02	0.74	0.72	0.04	0.0002	0.50	36.0
Middle	0.54	0.09	0.20	0.11	0.12	0.06	0.19	49.0
Leg	<.0001	0.09	0.87	0.47	0.76	0.91	0.65	32.4
Rigor mortis	-	0.05	0.17	-	-	0.27	-	-

Vocalization

Results for the main effects of vocalizations parameters are presented in Table 3. There was no effect ($P \geq 0.21$) of sex on any vocalization parameters. The mean frequency of all vocalizations ranged from 2504 to 2547 Hz, both sex and RAC treatment presented high frequency vocalizations, but no significant difference ($P = 0.21$) among treatments was detected. Fundamental frequency is the first and lower harmonic, and the mean values were also not affected ($P \geq 0.94$) by sex and RAC treatment. Differences in vocalization were observed in amplitude ($P < 0.0001$) and sound intensity ($P = 0.02$), RAC fed pigs presented higher values for both variables. There were no significant interactions among sex and RAC treatment for frequency ($P = 0.30$) and fundamental frequency ($P = 0.15$), however for amplitude and sound intensity a sex x RAC interaction was observed. Ractopamine treatment was responsible for higher amplitude in immunocastrates and gilts ($P < 0.0001$) when compare to pigs fed a control diet (Figure 3). Barrows were not influenced

(P > 0.05) by RAC (Figure 3). Immunocastrates fed RAC presented greater sound intensity than immunocastrates fed control diet (81 vs. 61 Hz; P < 0.02), while no differences (P > 0.05) were observed in barrows and gilts for RAC treatments (Figure 4).

Table 3. Least squares means and standard errors for main effects of sex and ractopamine treatment on vocalization

	Sex			Treatment	
Trait	Immunocastrate[1]	Barrow[2]	Gilt	Control	Ractopamine[3]
Sound intensity (dB)	71 ± 43.39	72 ± 16.27	69 ± 12.74	65 ± 65.34[a]	76 ± 40.55[b]
Amplitude (dB)	89.5 ± 0.025	90.3 ± 0.413	89.5 ± 0.43	85.8 ± 0.349[c]	92.4 ± 0.015[d]
Frequency (Hz)	2504 ± 69.33	2547 ± 86.56	2518 ± 69.55	2516 ± 76.58	2529 ± 76.71
Fundamental frequency (Hz)	1041 ± 721.13	1048 ± 113.77	1054 ± 86.64	1048 ± 1048	1046 ± 827.55

[1]Boars were immunocastrated by giving 2 doses of Vivax® (Pfizer Animal Health) 4 and 8 weeks before slaughter.
[2]Piglets were physically castrated at the first week of their lives.
[3]Ractopamine (Ourofino Agronegócio, Brazil) dose was 7.5 mg/kg for 21 d before slaughter.
[a,b]Within a row and main effect, least-squares means that do not have a common superscript letter differ (P < 0.05).
[c,d]Within a row and main effect, least squares means that do not have a common superscript letter differ (P < 0.01).

Blood parameters of stress

Results of mean values of blood physiological measurements, cortisol, CPK and LDH are presented in Table 4. There were no differences ($P \geq 0.08$) in cortisol and CPK concentrations measured between pigs fed the control or ractopamine diet, among sex and also between farms. However, gilts fed 7.5 mg/kg of RAC had greater cortisol concentrations than control diet (sex x RAC interaction; $P \leq 0.05$; Figure 5). No differences ($P \geq 0.37$) for RAC treatment and sex categories were detected in LDH concentration. Blood measurements for LDH from farm A ranged from 1105 to 7000 UI/L and were significantly higher (P < 0.01) than values from farm B, ranging from 540 to 1465 UI/L. When comparing farms within sex categories, an increased in LDH levels were also observed for all sexes from farm A (sex x farm interaction; P < 0.03; Figure 6).

Table 4. Least squares means and standard errors for main effects of farm, sex and ractopamine treatment on blood parameters of stress

	Farm		Sex			Treatment	
Trait	A	B	Immunocastrate[1]	Barrow[2]	Gilt	Control	Ractopamine[3]
Cortisol ng/ml	88.7 ± 24.26	98.5 ± 24.9	86.5 ± 25.99	91.4 ± 20.23	96.5 ± 27.2	86.9 ± 21.64	96.1 ± 26.91
CPK UI/L	9015 ± 4891	7639 ± 4237	8292 ± 4469	9332 ± 4923	7338 ± 4349	8257 ± 4674	8526 ± 4646
LDH UI/L	2063^{c} ± 499	854^{d} ± 268	1575 ± 761	1562 ± 814	1379 ± 568	1548 ± 722	1480 ± 741

[1]Boars were immunocastrated by giving 2 doses of Vivax® (Pfizer Animal Health) 4 and 8 weeks before slaughter.
[2]Piglets were physically castrated at the first week of their lives.
[3]Ractopamine (Ourofino Agronegócio, Brazil) dose was 7.5 mg/kg for 21 d before slaughter.
[c,d] Within a row and main effect, least squares means that do not have a common superscript letter differ (P < 0.01).

Hoof Lesions

After slaughter and exsanguination all animals were hung and their front hoof were evaluated for the presence or absence of individual lesions, such as splits, cracks-erosions, and bruises (Table 5). There was no evidence that RAC diet had any influence on incidence of hoof lesions on finishing pigs ($P \geq 0.09$). However, RAC fed pigs from farm B had twice as many the split lesions than control diet ($P < 0.01$; farm x RAC interaction; Figure 7). Additionally, a 3-way interaction was detected for bruise lesions ($P < 0.01$) and immunocastrates from farm B and barrows from farm A fed with ractopamine presented greater ($P < 0.01$) percentages of bruises lesions when compared with control (Table 6). On the other hand, RAC fed gilts from farm B had less ($P < 0.01$) incidence of bruises lesions (Table 6). When analyzing effects of sex on hoof lesions, it was found that physically castrates presented the highest percentage of crack-erosions, and immunocastrates showed more incidences of crack-erosions than gilts ($P < 0.01$). Bruise lesions from immunocastrated pigs were considerably higher than giltss ($P < 0.01$). Barrows did not differ from the other sex categories ($P > 0.05$). No differences were detected among sex for split lesions ($P = 0.56$). An interaction between farm and sex was detected ($P = 0.02$) and it was found that in farm A; immunocastrates had greater ($P < 0.05$) percentages of bruises than gilts and barrows (Figure 8), but for split lesions immunocastrates did not differ (*P* > 0.05) from the other categories (Figure 9). Gilts from farm A had more incidences of splits (*P* < 0.05) than barrows (Figure 9), but it did not differ for bruises lesions (Figure 9). In farm B, barrows presented greater percentages of splits and bruises than gilts (*P* < 0.05), and more splits than immunocastrated pigs (*P* < 0.05; Figure 8 and 9). In respect to the farm x sex x RAC interaction for bruises lesions some differences were found (*P* = 0.003; Table 6). Immunocastrated pigs from farm A - that received the control diet – had more lesions than barrows and gilts fed the control diet (*P* < 0.05). The exactly opposite occur in farm B, where immunocastrates had less (*P* < 0.05) bruise lesions. Barrows and immunocastrated pigs fed RAC, from farm B, presented more frequency of bruises than gilts (*P* < 0.05). In respect to differences between farms, the frequency of hoof lesions were greater in farm A, including splits and bruises (*P* < 0.05), with the exception of crack-lesions that were more incidente in farm B (*P* < 0.01). Immunocastrated pigs from farm A had more incidences of split, when compared to farm B (Farm x RAC interaction, *P* = 0.02; Figure 7) and gilts from farm A also presented greater percentage of split lesions than barrows (farm x RAC interaction, *P* = 0.02). A farm x sex x RAC interaction was detected (P < 0.003) for bruise lesions, and it was found some differences between farms. Immunocastrated fed the control diet and gilts fed RAC from farm A had more incidence of bruises (*P* < 0.05) . In contrast, farm B presented higher percentage of bruises for barrows fed the control diet (*P* < 0.05).

Table 5. Least squares means and standard errors for main effects of farm, sex, and ractopamine treatment on hoof lesions.

Trait	Farm		Sex			Treatment	
	A	B	Immunocastrate[1]	Barrow[2]	Gilt	Control	Ractopamine[3]
Split (%)	54 ± 0.036[a]	41 ± 0.051[b]	43 ± 0.056	51 ± 0.051	48 ± 0.057	42 ± 0.047	52 ± 0.041
Crack-erosion (%)	23 ± 0.039[c]	51 ± 0.048[d]	37 ± 0.050[a]	49 ± 0.051[b]	23 ± 0.056[c]	33 ± 0.051	39 ± 0.042
Bruise (%)	51 ± 0.038[a]	37 ± 0.050[b]	53 ± 0.058[a]	43 ± 0.051[ab]	36 ± 0.055[b]	38 ± 0.045	45 ± 0.045

[1]Boars were immunocastrated by giving 2 doses of Vivax® (Pfizer Animal Health) 4 and 8 weeks before slaughter.
[2]Piglets were physically castrated at the first week of their lives.
[3]Ractopamine (Ourofino Agronegócio, Brazil) dose was 7.5 mg/kg for 21 d before slaughter.
[a,b,c] Within a row and main effect, least-squares means that do not have a common superscript letter differ (P < 0.05).
[c,d] Within a row and main effect, least squares means that do not have a common superscript letter differ (P < 0.01).

Table 6. Least squares means and standard errors for farm x RAC x sex interaction effects for bruise lesions

	Sex	Farm A		Farm B	
		Control	Ractopamine[3]	Control	Ractopamine[3]
Bruise	Immunocastrate[1]	72 ± 0.074[Aaf]	67 ± 0.078[Aaf]	19 ± 0.097[Bbg]	58 ± 0.113[Aaf]
	Barrow[2]	22 ± 0.067[Bbg]	59 ± 0.078[Aaf]	42 ± 0.113[Aaf]	53 ± 0.121[Aaf]
	Gilt	35 ± 0.106[Abf]	53 ± 0.083[Aaf]	47 ± 0.114[Aaf]	15 ± 0.079[Bbg]

[1]Boars were immunocastrated by giving 2 doses of Vivax® (Pfizer Animal Health) 4 and 8 weeks before slaughter.
[2]Piglets were physically castrated at the first week of their lives.
[3]Ractopamine (Ourofino Agronegócio, Brazil) dose was 7.5 mg/kg for 21 d before slaughter.
[A, B] Within row, from each farm , means with different capital letters differ (P < 0.05)
[a, b] Within columns, means with different lowercase letters differ (P < 0.05)
[f, g] Comparing diferent farms columns with the same treatment, means with different letters differ (P < 0.05)

Skin Damage and Rigor mortis

After scalding and evisceration, carcasses were evaluated for early rigor development and skin damage score. The additon of RAC on the diet of finishing pigs did not affect the measurement of *rigor mortis* ($P > 0.05$; Table 7). Imunocastrated pigs fed RAC presented lower skin damage in the shoulder than immunocastrated fed control diet ($P < 0.01$; Figure 11). When evaluated the RAC x sex interaction, within control diet treatment immunocastrated pigs fed RAC had greater mean score for shoulder ($P < 0.01$; Figure 11) than barrows and gilts. No differences were observed for middle and leg lesions among sex ($P > 0.05$; Table 7). Barrows presented higher incidence ($P < 0.05$; Table 7) of rigor development when compared to gilts and immunocastrated pigs. Shoulder and middle lesions also did not present ($P \geq 0.18$; Table 7) differences between farms. On the other hand, animals from farm B showed higher incidence ($P < 0.01$) of leg lesions than animals from farm A. In farm x sex interaction the same results were found, but only for pigs fed the control diet (Figure 10). Pigs from farm B did not develop an early rigor in any of the 108 pigs analyzed; on the other hand 24% of rigor was found in animals from farm A (Table 7).

Table 7. Least squares means and standard errors for main effects of farm, sex, and ractopamine treatment on skin damage and *rigor mortis*.

	Farm				Sex						Treatment			
Trait	A		B		Immunocastrate[1]		Barrow[2]		Gilt		Control		Ractopamine[3]	
Shoulder	2.3 ±	0.491	2.4 ±	0.5493	2.5 ±	0.5386[a]	2.3 ±	0.4474[b]	2.4 ±	0.5331[ab]	2.4 ±	0.5138	2.4 ±	0.512
Middle	2.3 ±	0.479	2.3 ±	0.5185	2.4 ±	0.5287	2.3 ±	0.4413	2.3 ±	0.5017	2.3 ±	0.5084	2.3 ±	0.478
Leg	2.1 ±	0.3301[c]	2.4 ±	0.5029[d]	2.3 ±	0.4429	2.2 ±	0.386	2.2 ±	0.4138	2.2 ±	0.4103	2.2 ±	0.420
Rigor mortis (%)	24 ±	0.4289[a]	00 ±	0.000[b]	20 ±	0.8[b]	35 ±	0.95[a]	17 ±	0.75[b]	28 ±	0.78	20 ±	0.91

[1]Boars were immunocastrated by giving 2 doses of Vivax® (Pfizer Animal Health) 4 and 8 weeks before slaughter.
[2]Piglets were physically castrated at the first week of their lives.
[3]Ractopamine (Ourofino Agronegócio, Brazil) dose was 7.5 mg/kg for 21 d before slaughter.
[a,b,c] Within a row and main effect, least-squares means that do not have a common superscript letter differ (P < 0.05).
[c,d] Within a row and main effect, least squares means that do not have a common superscript letter differ (P < 0.01).

DISCUSSION

Vocalization

Most research has studied the piglets vocalization during castration (White et al., 1995; Weary et al., 1998; Marx et al., 2003; Puppe et al., 2005; Leidig et al. 2009), during nursing (Appleby et al., 1999), when submitted to social isolation (Colonnello et al., 2010), in adaptation to ambient temperatures (Hillmann et al., 2004) during human handling (Marchant et al., 2001; Grandin, 2001). Nevertheless, all these studies have been shown that vocalization can be an important indicator for stress condition of the farm animals, thus it can be an interesting non invasive tool to detect the possible impact of ractopamine on animal well-being. There is no evidence of a previous study with RAC that had evaluated its impact on pig vocalization.

In the present study immunocastrates, barrows and gilts had their vocalizations recorded during a stressful situation, although no significant differences were observed for any vocalization variables evaluated among sex it does not indicate that the animals were not in a negative state of welfare. Vocalizations were classified as high if their frequency was greater than 1.000 Hz; otherwise, they were classified as low (Weary et al., 1998). The addition of ractopamine in the diet also did no influence the frequency and the fundamental frequency of the pigs' vocalization. However, high frequencies were detected for all treatments; this result was expected once the vocalizations were recorded under intense stress condition for the animals, where they were submitted to human interaction during handling through a narrow corridor with limited social contact with the others pigs until the stunning box. Marx et al. (2003) classified piglets' vocalization during castration in: grunts, squeals and screams. In this study the vocalization of these pigs fit the description of grunts in relation to frequency. Usually piglets under castration procedure presented considerably number of squeals and screams, since these piglets are in extremely pain and their vocalization reach higher frequency calls (between 3200 to 5300 Hz) than pigs prior to slaughter. The vocalization of immunocastrated pigs fed RAC presented higher amplitude and greater sound intensity than control pigs. Gilts were also influence by ractopamine diet, and higher amplitude was detected when compared to control. Suggesting that ractopamine diet caused an important impact on animal vocalization and physically castrates pigs could be less susceptible to its influence. Moura et al. (2008) observed that pig's vocalization in stressful situations has an amplitude equivalent to 61.4

dB. In this study the all treatments amplitude range from 82.6 to 93.1 dB. The sound intensity found in this study is close to the mean values (71.4 ± 6.2 dB) for cow intensity of a single feed-anticipating call reported by Yeon et al. (2006).

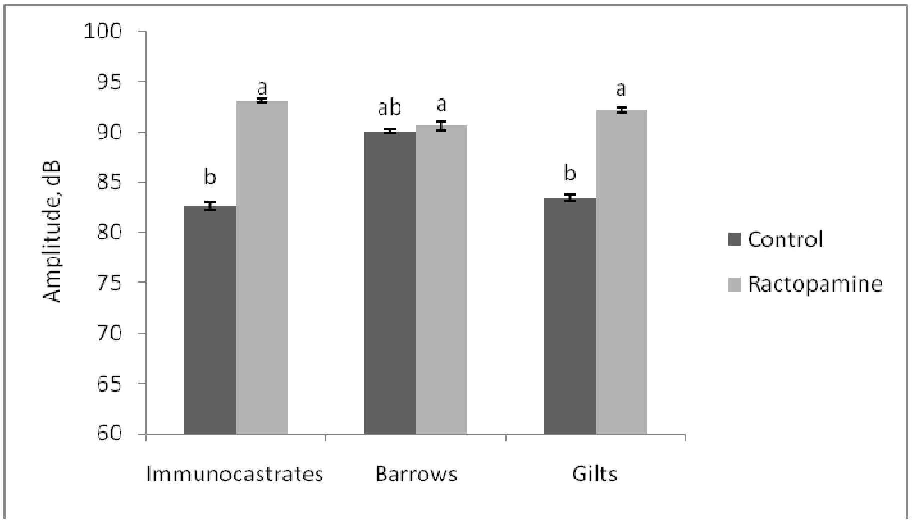


Figure 3. Sex x ractopamine interaction ($P < 0.01$) effects on amplitude and standard errors. Least squares means that do not have a common superscript letter differ ($P < 0.05$).

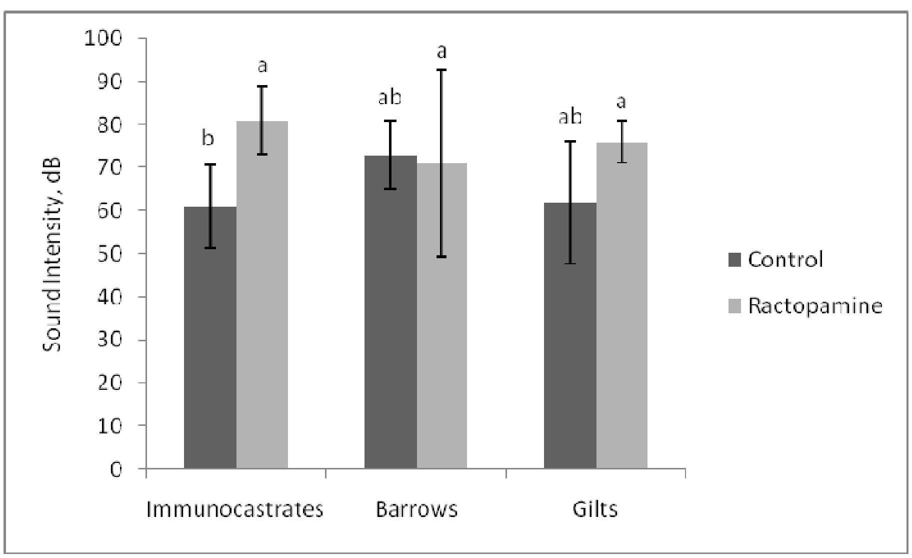


Figure 4. Sex x ractopamine interaction ($P < 0.01$) effects on sound intensity and standard errors. Least squares means that do not have a common superscript letter differ ($P < 0.05$).

Blood parameters of stress

In the present work, samples of blood were collected at exsanguination. The mean values of all blood variables investigated were expected to be elevated considering that at slaughterhouse pigs were exposed to physical exercise, mental influences, social changes and stunning. Thus changes in the blood profile may provide information on the stress of these pre-slaughter events. This fact does not influence the objective of detecting possible differences in the activity of hormones and enzymes induced by ractopamine, since all animal were submitted to the same stress conditions. Blood concentrations of lactate dehydrogenase (LDH), creatine phosphokinase (CPK), and cortisol are reported to be useful measures to indicated animal stress (Fábrega et al., 2004; Averos, 2007; Li et al., 2008). Gispert et al. (2000) used the measurement of blood cortisol to indicate that pigs were subjected to less stressful conditions in summer than in winter period. Chai et al. (2010) observed an increased in concentration of cortisol in pigs transport during long periods and under high density. The blood constituents' values presented high standard errors means, due to a great variability of blood samples (n = 10). The way animals react to stress conditions and the effect of these reactions on blood hormones and enzymes concentrations can widely vary, since each individual is unique and its possibly resulting stress levels are subjective and individual-dependent (Terlouw et al., 2005). In the present study, the physiological responses of animals to adverse conditions, such as handling and stunning, were not influenced by ractopamine, and sex condition did also not impact in changes of the blood measurements. Although cortisol concentrations did not differ between RAC fed pigs and control pigs, and also among sex condition, cortisol levels were elevated in all pigs compared with baseline cortisol concentrations of non-stressed pigs (Sutherland et al., 2008), suggesting that all pigs were experiencing stress prior to harvesting. In contrast with the sex condition results, Averos et al. (2007) observed greater cortisol values for physically castrate pigs than gilts. In the present study, a sex x RAC interaction was detected and gilts submitted to RAC diet presented higher cortisol concentrations, than control diet. It is an indication that gilts may have a more negative response to ractopamine than physically castrates and immunocastrates. Marchant-Forde et al., 2003 did not find a significant difference in circulating cortisol concentrations between RAC and control treatment either before or after female pigs transportation to the slaughterhouse.

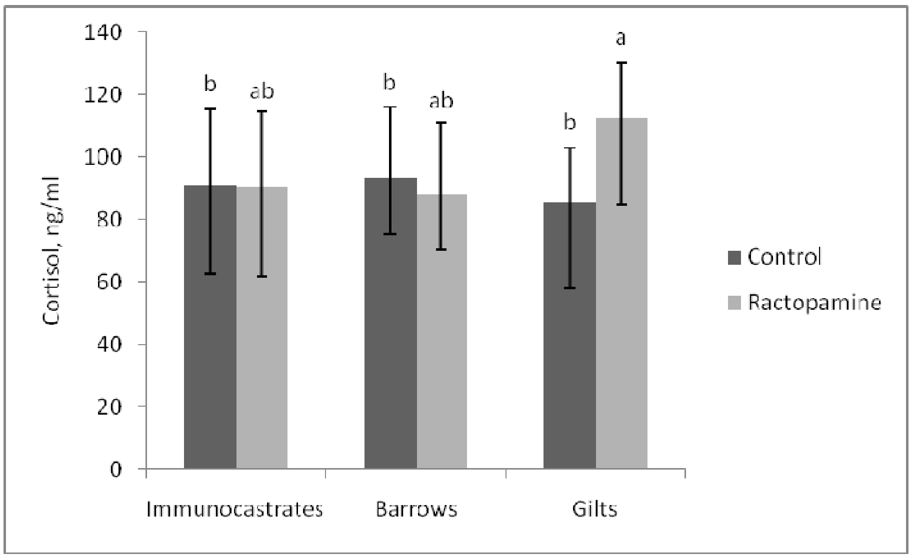


Figure 5. Sex x ractopamine interaction (P < 0.05) effects on cortisol and standard errors. Least squares means that do not have a common superscript letter differ (P < 0.05).

Creatine phosphokinase (CPK) and lactate dehydrogenase (LDH) are enzymes released from muscle fibers into blood in response to intense muscle exertion or tissue damage, it is commonly used as indicator of stress (Fàbrega et al., 2004; Sutherland et al., 2008). CPK and LDH values measured in this study were within an expected range for animals submitted to an acute stress (Averos et al., 2007). No significant differences were observed for CPK, among sex, between RAC diet and control, and between farms. In contrast, several authors reported increased of CPK concentrations under stress conditions, such as: high stocking density during transport (Warriss et al., 1998b; Chai et al., 2010), cold temperature (Gispert et al., 2000) and even an increase of CPK levels in pigs fed with the β agonist salbutamol (Warriss et al., 1990). In the present study, the enzymatic activity of LDH was also not affected by RAC diet, the strong individual variability in CPK and LDH could made differences not significant. Averos et al. (2007) evaluated LDH serum concentration in pigs transport to slaughter, and did not detect differences between gilts and barrows. This is in agreement with the present study that also did not find significant differences among sex condition. LDH concentrations were considerably higher in animals from farm A than farm B, an interaction between farm and sex was detected and also showed that all sex categories from farm A presented greater values for LDH than farm B. A possible explanation could be the difference between genetic background from the farms, since it is well known that different breeds or lines may vary in their stress reactivity. Although, other differences between farms should be taken into account, once animals were slaughter under different facilities and were handle by distinct people.

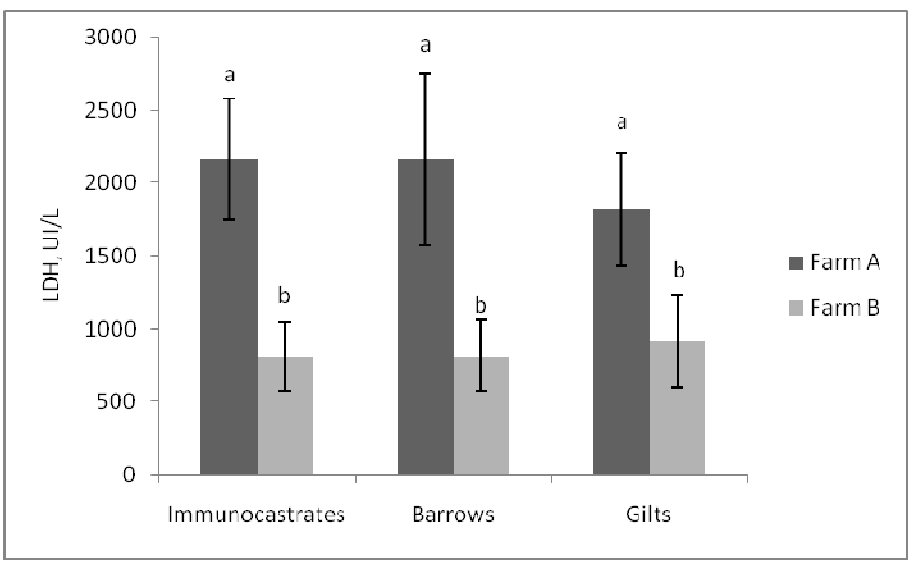


Figure 6. Farm x sex interaction (P = 0.03) effects on LDH and standard errors. Least squares means that do not have a common superscript letter differ (P < 0.05).

Hoof Lesions

The negative impact of ractopamine on the incidence of hoof lesions was noted in pigs from farm B, since RAC fed pigs presented twice as many splits that control pigs. And also the incidence of bruises increased specifically in immunocastrates (Farm B) and barrows (Farm A) submitted to RAC treatment. Negative effects on hoof soundness cause by ractopamine were also observed by Poletto et al. (2009). However, in this study the impact of ractopamine appeared only on the interactions, once no differences were observed for ractopamine, when the main effect were analyzed. In contrast, Poletto et al. (2009) reported that RAC fed pigs had notably more hoof lesions including crack-erosions and bruises and nearly twice as many overall hoof lesions compared with control. Penny et al. (1994) fed the pigs with a compound from the same category that RAC, a β-adrenergic salbutamol, and reported an increased in the frequency and severity of hoof lesions in finishing pigs. In general, seems like gilts presented less incidence of hoof lesions, however is difficult to indicate which sex category had more or less hoof lesions, once the frequency of different types of lesions varied widely within each farm among the sexes. Poletto et al. (2009) reported greater frequency of hoof lesions for barrows.When analyzing the effects of farms, it was found that a greater incidence of hoof lesions, including splits and bruises, were observed in farm A. However, farm B presented higher incidence of crack-erosions than farm A. In this study animals from farm A were heavier – average of 100 kg , than farm B – average of 100 kg, this factor could be an aggravated when associated with flooring structure. Pigs from both farms were housed in different farms with similar pens, on concrete solid floor with a space allowance of 1.0 m^2/pig. However, differences in the management practices and rough flooring conditions can influence a predisposition of finishing pigs to more severe lesions.

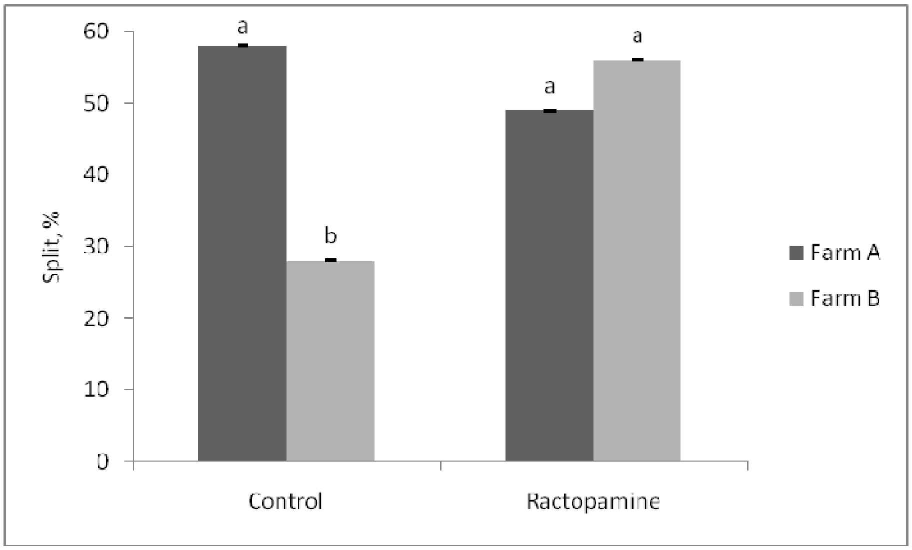


Figure 7. Farm x ractopamine interaction (P = 0.03) effects on split incidence and standard errors. Least squares means that do not have a common superscript letter differ (P < 0.05).

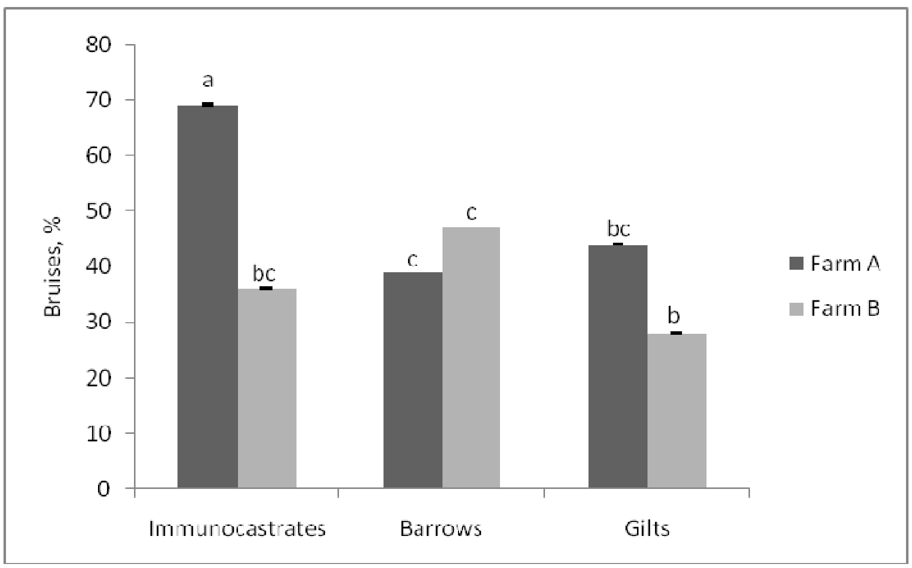


Figure 8. Farm x sex interaction (P = 0.02) effects on bruises incidence and standard errors. Least squares means that do not have a common superscript letter differ (P < 0.05).

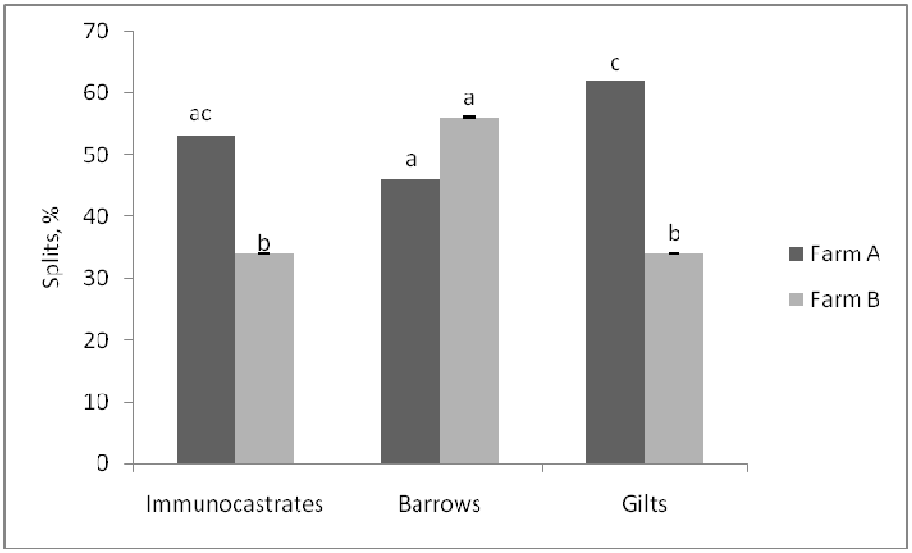


Figure 9. Farm x sex interaction (P = 0.03) effects on splits incidence and standard errors. Least squares means that do not have a common superscript letter differ (P < 0.05).

Skin Damage and Rigor

Skin damage is a serious commercial problem. The skin blemish score reflects the amount of fighting which pigs have indulged in preslaughter (Warriss et al., 1998a). The hypothesis for this measurement is that animals fed RAC can be more aggressive and hence more prone to fight. Thus, unfamiliar animals were not mixed during lairage in order to prevent fighting to establish a new social dominance order. Animals more susceptible to stress, can presente an earlier establishment of rigor mortis due to the reduction of muscle substrate stores (Warriss eta al., 1998a). In this study, the additon of ractopamine in the diet of finishing pigs did not present any negative influence on the incidence of skin damage and development of earlier *rigor mortis*; suggesting that these measurements did not indicate an increase on agressiveness of RAC fed pigs. In respect to sex, immunocastrated pigs fed the control diet had more incidence of skin damage on the shoulder than barrows and gilts. In general was also observed an increase in skin damage on the shoulder for immunocastrated pigs than barrows. It is likely that immunocastrated pigs fought more and for this reason had more occurrence of skin damage. Several authors have observed that entire males (Brown et al., 1999; Moss, 1978) are more aggressive due to their sexual behavior and had a higher occurrence of skin damage. Additionally, Guàrdia et al. (2009) found no evidence of an effect of sex, between barrows and gilts, on skin damage. However, no research evaluating the occurrence of skin damage on immunocastrated pigs was found in the literature. The incidence of early *rigor mortis* development was higher for barrows than immunocastrates and gilts. Animals from farm B had higher shoulder and skin damage average score, than pigs from farm A, the same result was found within pigs fed the control diet. The observed differences between farms for skin damage are multifactorial, with confounded and unknown factors, and so they are not easy to untangle. Guàrdia et al. (2009) have also shown the different effects among processing plants on the incidence of skin damage.

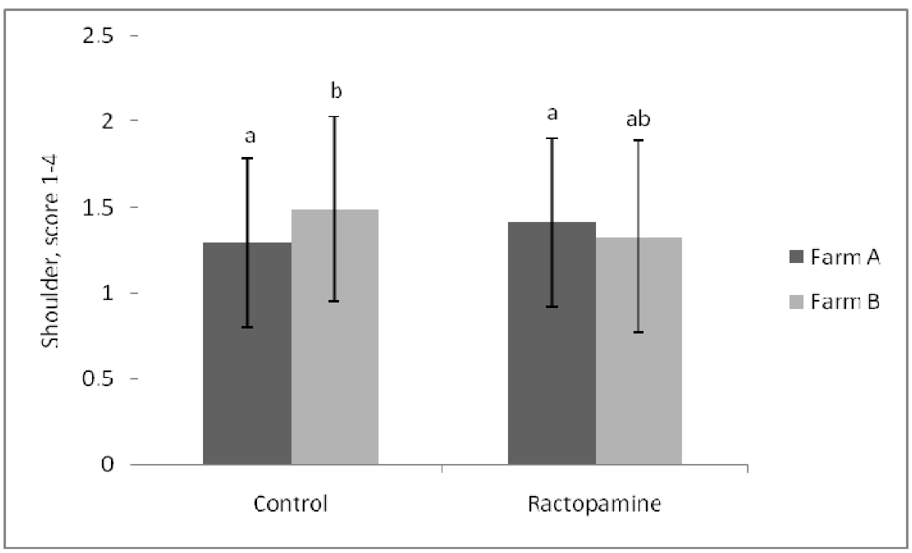
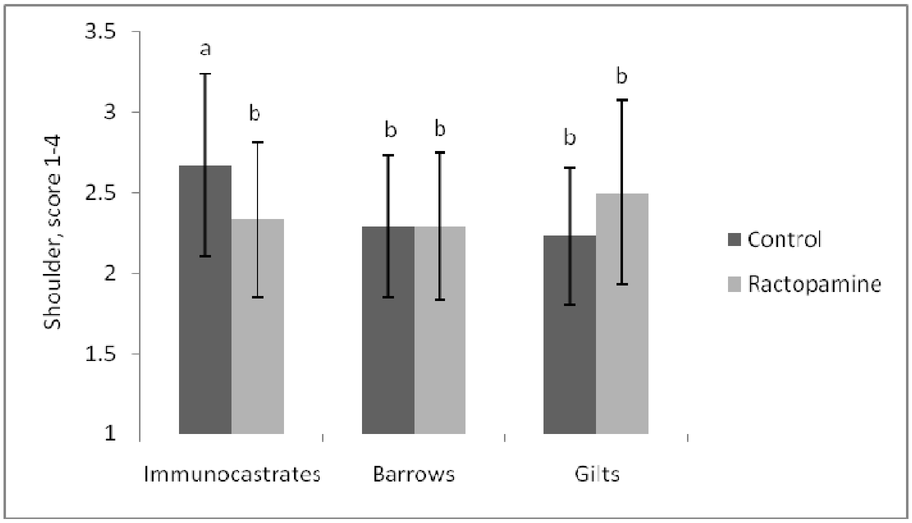


Figure 10. Farm x ractopamine interaction (P = 0.04) effects on skin damage of shoulder and standard errors. Least squares means that do not have a common superscript letter differ (P < 0.05).



11. Sex x ractopamine interaction (P = 0.0002) effects on skin damage of shoulder and standard errors. Least squares means that do not have a common superscript letter differ ($P < 0.05$).

CONCLUSION

The addition of ractopamine at 7.5 mg/kg on the diet of finishing pigs did not show an impact on LDH and CPK parameters of stress, skin damage and early development of *rigor mortis*. However, some vocalization parameters, hoof lesions and cortisol were greater for RAC fed pigs. In general, gilts presented lower values for the parameters of stress evaluated than immunocastrates or barrows. Is difficult to point with sex category were more susceptible to stress, once different results were observed for each sex in different stress parameters measured. The farms influence the incidence of many welfare parameters. Animals from farm A presented greater parameters of stress, including splits, bruises, early development of *rigor mortis* and especially LDH blood levels, than farm B. This difference can be associated to many factors, such as farm and slaughterhouse facilities, transport, handling and genetic background.

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ARTIGO 2: RACTOPAMINE HYDROCHLORIDE AND IMMUNOCASTRATION EFFECTS ON FRESH PORK PROPERTIES

O artigo a seguir está redigido de acordo com as normas para publicação na revista *Meat Science.*

Ractopamine hydrochloride and immunocastration effects on fresh pork properties

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ABSTRACT

Fresh pork propeties were evaluated in crossbred gilts, barrows and immunocastrated male pigs fed 7.5 mg/kg of ractopamine hydrochloride (RAC) for the final 21 d before slaughter. The research was conducted in two different farms. In farm A, pork samples originated from Topigs (Tempo sires × Topigs 40 dams) and samples from farm B were from Agroceres PIC pigs (AGPIC 337 sires × CB 22 dams). A total of 60 pork samples (n = 20/sex) from each farm were evaluated to access pork quality parameters. The pH_{24h} was determined on the M. *longissimus dorsi* (LD) and on the M. *semimembranosus* (SM) of carcasses, whereas the objective color, drip loss, cooking loss and Warner-Bratzler shear force (WBSF) were measured on the LD samples only. RAC fed pigs demonstrated greater ($P \leq 0.02$) pH_{24} (SM), cooking loss and shear force values than control pigs. No interaction of sex x RAC for any of the meat properties studied was detected ($P \geq 0.06$), suggesting that the combination of RAC and immunocastration has no impact on pork properties. Moreover, immunocastrated pigs did not differ ($P > 0.05$) from barrows for any pork properties studied. The incidence of RSE was extremely high, 80.0 % of the total samples evaluated presented this exudation problem, and 10.3 % were classified as PSE. These results were probably caused by poor pre-slaughter conditions in the slaughterhouses. Animals from farm A had higher pH_{24} (SM), cooking loss percentage, a* values for objective color ($P < 0.0001$) and shear force ($P < 0.0002$). Drip loss, L* and b* values of objective color were not affected ($P \geq 0.19$) by farms. RAC diet did

not affect ($P \geq 0.09$) objective color (L*a*b*), drip loss percentage (24 and 48 h) and pH_{24} (LD) of pork. RAC did not influence the incidence of PSE or RSE. It was concluded that, the sex condition and the addition of RAC on diet of finishing pigs had no important impact on fresh pork properties, evaluated in this research, with the exception of WBSF that was increased by RAC feeding.

INTRODUCTION

Ractopamine hydrochloride (RAC) is a β-adrenergic agonist added to finishing pigs diets to improve swine growth performance. This metabolic modifier acts as a repartioning agent, redirecting nutrients to increase protein deposition (Bergen et al., 1989) and to decrease adipose tissue accretion rate (Mills et al., 1990). RAC is structurally similar to epinephrine and norepinephrine and can be used to improve feed efficiency and carcass leanness (Uttaro et al., 1993; See et al., 2004; Weber et al., 2006; Marinho et al., 2007). Although the economic benefits of the use of RAC in pigs are well established there is a need to be aware of the possible changes that this additive can cause on pork quality. Stoller et al. (2003) and Carr et al. (2005) reported that feeding diets containing 10 mg/kg RAC for 25 to 41 days did not affect pork quality. On the other hand, in two previous studies, it was observed an increase in toughness in muscle of pigs fed 5 mg/kg RAC during 21 or 28 days (Stahl et al., 2007; Fernández-Dueñas et al., 2008).

Immunocastration is an alternative to the surgical castration, and a technology that has been developed to reduce boar taint compounds in pork. The vaccine temporary suppresses of testicular function through vaccination against gonadotrophin releasing hormone (GnRH) (Bonneau et al., 1994; Bonneau and Enright, 1995; Dunshea et al., 2001; McCauley et al., 2003; Oliver et al., 2003). Imunocastration does not affect pork properties (Gispert et al., 2010). However, previous studies reporting the effects of simultaneous use of immunocastration and ractopamine feeding on quality traits seem to be inexistent. Our hypothesis is that pigs fed RAC could present a greater stress response and it would reflect on the pork quality. Thus, the aim of this study was to evaluate fresh pork properties from pigs fed RAC and control diet, also taking into account sex categories with the objective to detect a possible interaction between imunocastration and RAC feeding, in two different farms with distinct genetic background.

MATERIAL AND METHODS

Animals

This study employed 310 finishing pigs (95 gilts, 107 immunocastrates and 108 barrows), weighing between 108–129 kg. Animals were housed in two different commercial farms representing distinct genetic crosses: Tempo sires × Topigs 40 dams from farm A (n = 202) and AGPIC 337 sires × CB 22 dams from farm B (n = 108). The experiment evaluated the impact of the inclusion of ractopamine hydrochloride (RAC; Ractosui®, Ourofino Agronegócio, Cravinhos, SP) at 7.5 mg/kg on the diet of gilts, barrows and immunocastrated pigs. The male piglets were physically castrated by tearing of tissue in their first week of life. They were raised and handled according to standard commercial procedures. They were housed in a naturally ventilated finishing barn on solid concrete floor with a space allowance of 1.0 m^2/pig. The pigs were provided with *ad libitum* access to a single spaced feeder and two nipple drinkers. The boars designated to be immunocastrated received their first vaccination dose (Vivax® Pfizer Animal Health, 2 mL) 8 weeks prior slaughter, and the second dose at 4 weeks prior slaughter. Ractopamine was included in the diet at an inclusion rate of 7.5 mg/kg, during 21 days (± 2 days) before slaughter. The conventional diet was based on corn and soybean, and the nutrition composition is showed in Table 1. At the end of the finishing period, pigs from farm B were transported, for approximately 10 km during 30 minutes to a commercial pork processing plant (Frigorífico Bressiani, Capivari, SP; plant B) and two weeks later pigs from farm

A were transported for approximately 250 km during four hours to a commercial pork processing plant (Frigorífico Mondelli, Bauru, SP; plant A). During transport, unfamiliar animals were mixed, but at the lairage pigs were penned with the same group they were housed in the commercial farm. At lairage, a stocking density of approximately 2.0 m^2/pig, in pens of 15–20 individuals was used; drinking water was available in the lairage pens. After an 8-h resting (plant A) and 12-h resting (plant B) period at the plant, animals were stunned with an electrical stunner (350–450V, 1–2A). Humane slaughter was conduct in accordance with the Brazilian Sanitary and Industrial Inspection Regulation for Products of Animal Origin (Brasil, 1997). Before chilling, 60 carcasses of each farm were selected according to hot carcass weight (HCW) and measures of carcass 10th-rib fat and *longissimus* muscle (LM) depths with a pistol Henessy probe (Hennessy Grading System) inserted between the 10th and 11th ribs. After 24 h in the chiller at 4 °C, the left side of each carcass was transported to the Meat Technology Center of the Institute of Food Technology for pork properties measurements.

Table 1. Nutritional composition of the finishing diet of the Farm A and Farm B.

		Farm A		Farm B	
		Control diet	RAC diet	Control diet	RAC diet
Metabolizable Energy	Kcal/kg	3,320.75	3,372.05	3,248.51	3,268.52
Net Energy - Swine	Kcal/kg	2,420.00	2,440.00	2,400.00	2,400.00
Crude Protein	%	15.00	17.00	15.00	17.00
Digest. Isoleucine	%	0.51	0.6	0.53	0.63
Digest. Lysine	%	0.75	0.95	0.85	0.95
Digest. Methionine	%	0.22	0.28	0.2	0.27
Digest. Meth + Cys	%	0.46	0.55	0.39	0.49
Digest. Threonine	%	0.48	0.62	0.55	0.62
Digest. Tryptofan	%	0.13	0.15	0.13	0.16
Crude Fiber	%	2.65	2.8	2.67	2.88
Soluble Fiber (A. D.)	%	3.45	3.73	5.24	5.41
Soluble Fiber (N. D.)	%	10.71	10.68	9.45	9.54
Starch	%	48.41	44.47	48.41	44.47
Total fat	%	3.76	4.73	3.26	3.68
Ash	%	3.55	3.78	4.44	4.45
Total Ca	%	0.60	0.60	0.70	0.60
Available P	%	0.39	0.37	0.32	0.31
Digestible P	%	0.3	0.29	0.25	0.25
Cl	%	0.43	0.45	0.42	0.44
Na	%	0.25	0.25	0.21	0.22
K	%	0.56	0.65	0.45	0.5

Properties Measurements

Loins (M. *longissimus dorsi;* LD) were evaluated for pH at 24 h *postmortem*, drip loss, cooking loss, Warner Bratzler shear force (WBSF) and objective color. At 24 h *postmortem*, instrumental color values were obtained from the LD using a Minolta Chromameter (CR-400; Konica Minolta Sensing, Inc., New Jersey, USA) with illuminant D65 and a 10° observer angle. The results were expressed as CIE units: L* (lightness); a* (redness); b* (yellowness). The pH_{24} was measured in M. *semimebranosus* (SM) using a Meat pHmeter (Hanna, Model H199163, Woonsocket, RI, USA) and also in the LD in pigs from farm A. The 1^{st}–10^{th} rib section of the LD muscle was excised and sliced into a 2.54-cm-thick chop for further Warner-Bratzler shear force analysis. The pork chops were weighed and cooked on a clamshell style grill (Sirman, Model PDL Snack, São Paulo, SP) to an internal temperature of 72 °C, which was monitored with a Food Thermometer (Testo, Model 106) inserted into the geometric center of each chop. Samples were then dried with paper towels to remove excess moist and reweighed to determine cooking loss expressed as a percentage of the initial sample weight (Honikel, 1998). An average of eight 1.27 cm diameter cores was removed from each chop parallel to the longitudinal orientation of the muscle fibers. Each core was sheared with a 3 mm thick Warner–Bratzler shearing device (Model TA.XT2i, Texture Analyser, Goldaming, Surrey, England) with a probe travel distance of 30 mm from the base, a pre-test speed of 5 mm/s, a test speed of 2 mm/s and a post-test speed of 5 mm/s. For drip loss, meat samples of 80 to 100 g were cut from the LD muscle at 24 h *postmortem* and immediately weighed (initial weight for drip loss). Samples were placed in the netting and suspended in an inflated bag, ensuring that samples did not have contact with the bag, and stored at 4° C. After 48 h storage period, samples were then removed from the bag, gently dried out with paper towel and weighed. The percent change in weight over the subsequent period was taken as the drip loss, as described by Honikel (1998). Drip loss was expressed as percentage of the initial sample weight. The drip loss percentage of pork samples from farm A was also determined after 24 h storage period.

To establish a criterion for pork quality determination, five categories according to measurements of color (L*), pH_{24} and drip loss (48 h) were used: PSE - Pale, Soft, and Exudative; RSE - Red, Soft, Exudative; DFD - Dark, Firm, and Dry, RFN - Red, Firm and Non-exudative, and PFN - Pale, Firm, Non-exudative (Meat Evaluation Handbook, 2001; Warner et al., 1997), as showed in Table 2.

Table 2. Pork quality classification adapted from Warner et al. (1997)

Classification	L*	Drip loss	pH_{24}
PSE[1]	> 50	> 5%	< 6
RSE[2]	42-50	> 5%	< 6
RFN[3]	42-50	< 5%	< 6
DFD[4]	< 42	< 5%	≥ 6
PFN[5]	> 50	< 5%	< 6
NC[6]	> 42	> 5%	≥ 6

[1]PSE - Pale, Soft, Exudative
[2]RSE - Red, Soft, Exudative
[3]DFD - Dark, Firm, Dry
[4]RFN - Red, Firm, Non-exudative
[5]PFN - Pale, Firm, Non-exudative
[1]NC – not classified in any of the pork quality categories

Statistical Analysis

The experiment was carried out as a factorial arrangement of 2 x 2 x 3 with the main effects of farm (Farm A vs. Farm B), RAC level (0 vs. 7.5 mg/kg) and sex condition (immunocastrates vs. barrows vs. gilts). The experimental design for carcass evaluation included a total of 60 samples (individual carcass = experimental unit) and 10 samples per treatment by set interaction. Data were analyzed by ANOVA using the GLM procedure of SAS (SAS Inst., Inc., Cary, NC) with a model including the main effects of RAC, sex, and farm, as well as the 2- and 3-way interactions. Least squares means were computed for main and interactive effects and were separated statistically using *F*-test (PDIFF). Tukey test ($p < 0.05$) was applied for the variables that showed difference. The incidence of pork quality categories (PSE, DFD, RSE, PFN and RFN) was analyzed by a nonparametric chi-square test, and statistical differences were declared at $P < 0.05$ level.

RESULTS

No interactions for any of the meat properties studied were detected ($P \geq 0.06$) as showed in Table 3. Therefore, only the main effects of farm, sex and RAC are presented in Table 5 and 6.

Table 3. P-values and coefficient of variation (CV) for all measurements evaluated by farm (F), sex (S) and ractopamine (R) effects

	P-value							
	F	S	R	F x S	F x R	S x R	F x S x R	CV (%)
Ligthness (L*)	0.71	0.79	0.41	0.98	0.25	0.89	0.45	4.21
Redness (a*)	<.0001	0.002	0.34	0.86	0.32	0.77	0.53	15.65
Yellowness (b*)	0.19	0.01	0.09	0.87	0.36	0.98	0.23	69.79
pH_{24h} (SM)	<.0001	0.01	0.02	0.13	0.76	0.34	0.64	3.11
Drip loss 48h, %	0.95	0.08	0.66	0.8	0.18	0.95	0.51	26.84
Cooking loss, %	<.0001	0.28	<.0001	0.09	0.09	0.49	0.82	12.91
Shear force, kg	0.0006	0.32	<.0001	0.06	0.16	0.42	0.73	14.23

Animals from farm A had higher pH_{24} (SM), cooking loss percentage (Table 6), a* values for objective color ($P < 0.0001$; Table 5) and shear force ($P = 0.0006$; Table 6). Animal from farm B presented greater ($P < 0.05$) incidence of RSE meat (86.4%), than animals from farm A (74.1%), as shown in Table 4. The others pork quality categories did not show significant difference ($P > 0.05$) between farms. Drip loss, L* and b* values of objective color were not affected ($P \geq 0.19$) by farms. Sex condition did influence objective color, a* and b* values, and pH_{24} (SM) ($P \leq 0.01$). Gilts presented higher pH values (5.71) than immunocastrates (5.59), but barrows (5.63; $P > 0.05$) did not differ from the others. Loin muscle from barrows and immunocastrates presented redder appearance (a* values) than loins from gilts ($P = 0.002$). Barrows also presented higher b* values than gilts, but immunocastrates did not differ from either subgroups ($P > 0.05$). The LD lightnesss had no significant (P = 0.79) difference among sex. Others important pork quality measurements, such as drip loss (48 h), cooking loss and shear force were unaffected (P ≥ 0.08) by sex condition. The analysis of drip loss (24 h) and pH_{24} (LD) carried out only in farm A, were also not influenced (P ≥ 0.11) by sex condition. The frequency of PSE was not significantly different (P > 0.05) among sex, however gilts had greater incidence (P<0.05) of DFD than barrows and immunocastrates (Table 4). Results from the present experiment demonstrated that the inclusion of 7.5 mg/kg of RAC in the diet of finishing pigs, did not affect (P ≥ 0.09) objective color (L*a*b*), drip loss percentage (24 and 48 h) and pH_{24} (LD). Loin chops from RAC fed pigs (5.7) showed greater (P < 0.001) pH_{24} (SM) than those from the controls (5.6). RAC diet did not increase the incidence of PSE meat (P > 0.05). The *longissimus dorsi* from pigs fed RAC had greater (P < 0.0001) shear force values and cooking loss percentage (3.5 kg and 24.6%, respectively) than control (3.1 kg and 22.4%, respectively).

Table 4. Incidence of each pork quality classification

Classification, %	Farm		Sex			Treatment	
	A	B	Immunocastrate[1]	Barrow[2]	Gilt	Control	Ractopamine[3]
PSE	12.1	8.5	10.3	10.0	10.5	11.7	8.8
RSE	74.1[b]	86.4[a]	79.5	82.5	76.3	80.0	80.7
RFN	6.9	3.4	2.6	5.0	7.9	5.0	5.3
DFD	0.0	1.7	0.0[b]	0.0[b]	2.6[a]	0.0	1.8
PFN	0.0	0.0	0.0	0.0	0.0	0.0	0.0
NC[4]	5.2[a]	0.0[b]	2.6	2.5	2.6	3.3	1.8

[1]Boars were immunocastrated by giving 2 doses of Vivax® (Pfizer Animal Health) 4 and 8 weeks before slaughter.
[2]Piglets were physically castrated at the first week of their lives.
[3]Ractopamine (Ourofino Agronegócio, Brazil) dose was 7.5 mg/kg for 21 d before slaughter.
[4]NC – not classified in any of the pork quality categories
[a,b]Within a row, least-squares means that do not have a common superscript letter differ (P < 0.05).

Table 5. Means of pork properties for objective color by sex, ractopamine and farm effects

	Sex			Treatment		Farm	
	Immunocastrate[1]	**Barrow[2]**	**Gilt**	**Control**	**Ractopamine[3]**	**A**	**B**
Ligthness (L*)	47.80 ± 1.79	47.94 ± 2.05	47.94 ± 2.07	47.94 ± 1.73	47.64 ± 2.16	47.85 ± 2.07	47.72 ± 1.84
Redness (a*)	6.71 ± 1.28[a]	6.87 ± 1.05[a]	6.10 ± 0.91[b]	6.64 ± 1.1	6.48 ± 1.17	6.98 ± 0.94[a]	6.13 ± 1.16[b]
Yellowness (b*)	1.36 ± 0.92[ab]	1.46 ± 0.88[a]	0.90 ± 0.8[b]	1.38 ± 0.87	1.11 ± 0.9	1.14 ± 0.85	1.36 ± 0.94

[1]Boars were immunocastrated by giving 2 doses of Vivax® (Pfizer Animal Health) 4 and 8 weeks before slaughter.
[2]Piglets were physically castrated at the first week of their lives.
[3]Ractopamine (Ourofino Agronegócio, Brazil) dose was 7.5 mg/kg for 21 d before slaughter.
[a,b]Within a row, least-squares means that do not have a common superscript letter differ ($P < 0.01$).

Table 6. Means of pork properties for pH_{24h}, drip loss, cooking loss and Warner Bratzler shear force by sex, ractopamine and farm effects

	Sex			Treatment		Farm	
	Immunocastrate[1]	**Barrow[2]**	**Gilt**	**Control**	**Ractopamine[3]**	**A**	**B**
pH_{24h} (SM)	5.59 ± 0.23[b]	5.63 ± 0.23[ab]	5.71 ± 0.26[a]	5.61 ± 0.22[c]	5.68 ± 0.26[d]	5.80 ± 0.18[a]	5.48 ± 0.19[b]
pH_{24h} (LD)	5.38 ± 0.27	5.39 ± 0.24	5.40 ± 0.28	5.38 ± 0.21	5.39 ± 0.27	- -	- -
Drip loss 24h, %	5.99 ± 2.34	6.21 ± 2.25	4.89 ± 2.32	5.38 ± 2.27	5.39 ± 2.22	- -	- -
Drip loss 48h, %	8.84 ± 2.18	8.84 ± 2.17	7.82 ± 2.34	8.60 ± 2.11	8.41 ± 2.42	8.50 ± 2.34	8.52 ± 2.21
Cooking loss, %	24.06 ± 4.11	23.13 ± 3.24	23.29 ± 3.48	22.39 ± 3.79[b]	24.62 ± 3.08[a]	25.00 ± 2.64[a]	22 ± 3.86[b]
Shear force, kg	3.42 ± 0.65	3.30 ± 0.43	3.29 ± 0.50	3.15 ± 0.49[b]	3.51 ± 0.52[a]	3.48 ± 0.47[a]	3.19 ± 0.56[b]

[1]Boars were immunocastrated by giving 2 doses of Vivax® (Pfizer Animal Health) 4 and 8 weeks before slaughter.
[2]Piglets were physically castrated at the first week of their lives.
[3]Ractopamine (Ourofino Agronegócio, Brazil) dose was 7.5 mg/kg for 21 d before slaughter.
[a,b]Within a row, least-squares means that do not have a common superscript letter differ ($P < 0.01$).
[c,d]Within a row, least-squares means that do not have a common superscript letter differ ($P < 0.05$).

DISCUSSION

There was no interaction between sex and RAC treatment; this is an important finding, once it shows that the combination of RAC diet and immunocastration had no impact on pork quality. To determine pork quality, all meat samples were classified in five categories: PSE, DFD, RSE, PFN and RFN; according to measurements of color (L*), pH_{24} and drip loss (48 h). The incidence of RSE was significantly higher for animals from farm B, however both farms presented extremely high percentage of this exudation problem. Eighth percent of the total samples analyzed were consider RSE meat and only 5.3 % RFN meat. The distribution of RSE among the treatments was homogeneous, thus it cannot be explained by farm, sex or RAC influence. The incidence of DFD was higher for gilts than barrows and immunocastrates, once only one pork sample from gilts was found with this abnormality, representing 2.6 %. No difference between the two castrations methods were found in this study, which suggests that immunocastration has no impact on pork quality. The incidence of PSE considered acceptable in Brazilian slaughterhouses is around 3 to 5 %. PSE meat was found in 10.3 % of the total samples analyzed, DFD only 0.9 % and PFN characteristics were not detected. PSE meat is the result of a rapid post-mortem glycolisis, resulting in extensive myofibrillar protein desnaturation, pale color and low water holding capacity (Van Laack, 1999). The presence of PSE is associated to an intense stress just before slaughter and to genetic background. The origin of RSE meat is unkown, however this meat defect is considered a mild form of PSE (Van Laack, 1999). RSE is associated with a moderately fast post-mortem glycolisis, but the myofibrilar protein denaturation of RSE meat is comparable to that of RFN meat. The only difference between normal and RSE meat is that denaturation of sarcoplasmatic proteins phosphorylase and creatina kinase is enhanced in RSE pork (Warner, 1994). The reason for this increased rate of glycolisis in RSE carcasses still needs to be determined. A possible hyphotesis is that RSE quality results from moderate stress before slaughter (Van Laack, 1999). In this project, the ramp angle for unloading exceed 20 degrees, in consenquence was observed several animal slipping during this process; moreover, electrical prod was also used during the pre-slaughter handling of the animals, in both slaughterhouses. Thus, it could be suggested that the handling conditions of pre-slaughter was the main cause of the high incidence of RSE and PSE meat, once it is known that the use of electrical prod and inadequate ramps has a negative impact on animal welfare and meat quality.

The most important parameter of the objective color for pork quality is the lightness. In this study RAC did not influence the L* values of pork, these results are in agreement with Carr et al. (2005), Sthal et al. (2007), Armstrong et al. (2004) and Gonzales et al. (2010). Gilts presented lower values of a* than barrows and immunocastrates, it indicates a reduction of oxymioglobin. For b* values, barrows presented greater results than gilts, but both did not differ from immunocastrates. Gispert et al. (2005) and Uttaro et al. (1993) found no difference for a* values between sex, however for b* values the study conducted by Uttaro et al. (1993) is in agreement with the present research. Despite the significant difference among sex for objective color a* and b*, the mean values for both variables are within an acceptable range for pork color reported by Sthal et al. (2007) and Apple et al. (2008). Some pork properties were incresead in loins from farm A. The pH 24 h (SM) from farm A was significantly greater than farm B. Cooking loss, shear force and a* values were also increased in loins of pigs from farm A. Stunning methods and chilling systems were the same in both processing plants where animals from farm A and B were slaughtered, thus only pre-slaughter factors such as genetic background of the animals, transport and handling should be considered. In this study RAC supplementation tended to increase final pH in SM muscle. In contrast with the findings of this study, RAC treatment does not commonly increase the pH_{24h} (Carr et al., 2005; Sthal et al., 2007; Weber et al., 2006). Despite this differences between RAC treatments, this alteraction in pH_{24} (SM) did not caused any impact on pork quality, once the mean values found are within a normal range of pH_{24} (Faucitano et al. 2010). Gilts presented greater pH_{24} values than immunocastrates, but barrows did not differ from both. Stoller et al. (2003) also found no difference in pH_{24h} values from gilts and barrows, but immunocastrated pigs were not analyzed. The low values of pH_{24h} on the LD and the high percentage of drip loss for all samples evaluated are characteristic of RSE and PSE meat.

Ractopamine treatment increased cooking loss of LD muscle. However, cooking loss is not a characteristic that is often increased by the supplementation of ractopamine (Stoller et al., 2003; Sthal et al., 2007; Apple et al., 2008; Fernández-Dueñas et al., 2008).Warner–Bratzler shear force analysis indicated that pork tenderness was negatively affected by RAC supplementation. The increase in shear force values in samples from RAC fed pigs can be a consequence of the higher cooking loss values found for RAC treatment. However, previous studies also reported an inverse relationship between dietary RAC supplementation and meat tenderness (Uttaro et al., 1993; Aalhus et al., 1998; Stahl et al., 2007; Fernandez-Dueñas et al. 2008). Fernández-Dueñas et al. (2008) also reported higher shear force values of meat from RAC fed pigs; however trained sensory panel were unable to detect this difference. Xiong et al. (2006) reported that the decrease in tenderness of meat from pigs fed ractopamine is likely due to the higher calpastatin activity and lower calpain activity in muscle. These results also demonstrated that the aging period must be taken into account, since the effect of ractopamine on the increased in shear force values decreased over time and were equal to the control group on the tenth day of ageing. Xiong's study concludes that *postmortem* ageing appears to be an effective means to tenderize pork from RAC fed pigs. The decreased of tenderness in pigs fed RAC can be also caused by the more cross-linked collagen than normal (Dawson et al., 1990 in Lawrie and Ledward, 2006). Additionally, Aalhus et al. (1992) suggested that ractopamine increased muscle fibers diameters. The larger diameter fiber can be associated with decrease of tenderness independent of connective tissue strength or age (Swatland, 1984).

CONCLUSION

No interaction between sex and RAC treatment was found, this is an important finding once it shows that the combination of RAC diet and immunocastration had no impact on pork quality. Animal sex condition had no important influence on fresh pork properties. Immunocastrated pigs did not differ from barrows. RAC diet did not increase the incidence of PSE and RSE meat. Regardless treatment, 80.0 % of all samples presented RSE meat problem and 10.3 % presented PSE meat, suggesting an improvement in the pre-slaughter conditions of the both slaughterhouses. The addition of RAC on diet of finishing pigs did increase cooking loss and WBSF toughness.

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CONCLUSÃO GERAL

A adição de ractopamina na dieta de suínos, na fase de terminação, não alterou os parâmetros fisiológicos de estresse sanguíneo, o incidência de lesões de pele e casco, e nem acelerou o desenvolvimento de *rigor mortis* na carcaça. No entanto, houve aumento da intensidade e amplitude da vocalização dos imunocastrados e fêmeas que consumiram a ração contendo este composto, a ractopamina parece não ter influenciado o estresse dos animais castrados cirurgicamente. Diversas interações entre ractopamina, condição sexual e granja foram observadas. A concentração de cortisol foi superior em fêmeas que consumiram ractopamina em comparação com fêmeas que receberam a dieta controle. Ao analisarmos a condição sexual independente da ractopamina, de uma forma geral as fêmeas apresentaram valores mais baixos para os índices de estresse avaliados, em comparação com castrados cirurgicamente e imunocastrados. Não foi possível eleger qual condição sexual é mais susceptível ao estresse, uma vez que as medidas realizadas não apontaram uma tendência definida. Não foi observada interação entre ractopamina e condição sexual, o que sugere que a combinação de ractopamina com imunocastração não afeta as propriedades da carne suína. A ractopamina não afetou índices de PSE e SER na carne, no entanto aumentou significativamente as perdas por cocção e os valores de WBSF.

A diferença entre as granjas onde os animais foram criados influenciou vários parâmetros de bem-estar animal e também algumas propriedades da carne suína. Animais criados na granja A apresentaram maiores índices de estresse e valores superiores das propriedades da carne, incluído pH_{24}, cor objetiva (a*), perdas por cocção, aumento na textura, maior incidência de lesões de casco (hematomas e rachaduras), desenvolvimento precoce de *rigor mortis* e aumento nos níveis sanguíneos de LDH, quando comparados a animais criados na granja B. Estas diferenças podem estar associadas a diversos fatores, como estrutura da granja e abatedouro, transporte, manejo, contato com humanos e principalmente a linhagem genética dos suínos. Isto sugere estudos mais aprofundados para comparação de linhagens em relação à ractopamina.