BRUNO REIS CARNEIRO DA COSTA LIMA

FRESH PORK AND PROCESSED PRODUCT QUALITY FROM RACTOPAMINE-FED AND IMMUNOCASTRATED PIGS

QUALIDADE DA CARNE E DE PRODUTO PROCESSADO PROVENIENTES DE SUÍNOS IMUNOCASTRADOS E ALIMENTADOS COM RAÇÃO CONTENDO RACTOPAMINA

Tese apresentada ao Programa de Pós-Graduação em Medicina Veterinária da Universidade Federal Fluminense, como requisito parcial para obtenção do grau de Doutor. Área de Concentração: Higiene Veterinária e Processamento Tecnológico de Produtos de Origem Animal.

Orientador: Prof. Dr. TEÓFILO JOSÉ PIMENTEL DA SILVA

Coorientador: Prof. Dr. EXPEDITO TADEU FACCO SILVEIRA (*In memoriam*)

Niterói

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Aprovado em:

BANCA EXAMINADORA

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Prof. Dr. Teófilo José Pimentel da Silva—Orientador
Universidade Federal Fluminense

_____

Prof. Dr. Anderson Junger Teodoro
Universidade Federal do Estado do Rio de Janeiro

_____

Prof. Dr. Surendranath P. Suman
University of Kentucky

_____

Prof. Dr. Walter Lilenbaum
Universidade Federal Fluminense

_____

Prof. Dr. Thiago da Silveira Alvares
Universidade Federal do Rio de Janeiro

_____

Prof. Dr. Carlos Adam Conte Júnior
Universidade Federal Fluminense

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RESUMO

A indústria da carne suína utiliza diversas alternativas para melhorar tanto a qualidade e a quantidade de carne produzida quanto, controlar defeitos tecnológicos. Com a finalidade de aumentar a produção de carcaças com alto teor de carne magra, são utilizados aditivos alimentares como a ractopamina. Este agonista beta-adrenérgico promove o aumento de carne magra através do estímulo do anabolismo protéico em detrimento da lipogênese. Além disso, outro aspecto de grande importância na suinocultura é o controle do odor sexual na carne de suínos machos causado pelo acúmulo de androstenona (esteróide testicular) e escatol (metabólito da microbiota intestinal) no tecido adiposo. O método de rotina para o controle deste defeito é a castração cirúrgica de leitões; porém tal procedimento potencialmente promove o aumento da morbidade e mortalidade no plantel. Como alternativa utiliza-se a imunocastração, tecnologia que estimula o desenvolvimento de resposta autoimune contra o fator liberador de gonadotrofinas (GnRF) e consequentemente promove a atrofia dos testículos. Embora existam diversos estudos investigando a influência destas técnicas sobre a qualidade da carne fresca, o conhecimento de seus efeitos em nível molecular e sobre a qualidade do produto cárneo processado é escasso. Sendo assim, objetivou-se investigar os efeitos da adição de ractopamina em dieta suína e da imunocastração sobre os parâmetros de qualidade de produto emulsionado (salsicha; artigo 1). A partir dos resultados do artigo 1 pode-se concluir que a ractopamina não promoveu efeitos negativos sobre a qualidade da salsicha, e exerceu efeito sexo-específico sobre os parâmetros analisados. Salsicha elaborada a partir do pernil oriundo de macho castrado cirurgicamente apresentaram parâmetros diferentes dos produtos elaborados a partir de pernil de fêmea e de macho imunocastrado. Como a finalidade de compreender em base molecular, os efeitos da adição de ractopamina na dieta suína sobre a qualidade da carne, foi comparado o perfil sarcoplasmático do lombo suíno (*Longissimus thoracis*) de animais alimentados com dieta contendo ractopamina, com animais alimentados com dieta sem o aditivo (artigo 2). A partir da análise proteômica, observou-se que a alimentação com ractopamina altera a abundância de determinadas enzimas glicolíticas bem como, aumenta a abundância de proteínas relacionadas com a regulação da lipogênese. Isto posto, conclui-se que o aumento do teor de carne magra é promovido pela inibição da lipogênese bem como, o incremento na relação proteina/gordura deve ser explorado pela indústria cárnea uma vez que não afeta negativamente a qualidade do produto final.

Palavras-chave: Proteoma; Lombo suíno; SDS-PAGE; Imunocastração; Beta agonista

ABSTRACT

The pork industry utilizes several technologies aimed at improving the quality and quantity of meat produced, and minimizing/preventing adverse meat defects. Some of these tools, like the food additive ractopamine, are focused on increasing the amount of carcass leanness produced. This beta-adrenergic agonist promotes the increase of lean meat by stimulating protein anabolism rather than lipogenesis. Another main focus in swine production is the prevention of boar taint in meat; this undesirable characteristic is caused by an accumulation of androstenone (testicular steroid), and skatole (metabolite of intestinal bacteria) in the adipose tissue. The standard prevention method is to surgically castrate the piglets, which potentially increases both the morbidity and the mortality rates. An alternative method is to immunocastrate the animal, which promotes the development of an autoimmune response against the Gonadotropin-releasing factor (GnRF) and consequently the development of testicular atrophy. Although there are many studies investigating the influence of these technologies on the quality of fresh pork, the literature on their effects at the molecular level and on the quality of processed meat that is produced thereafter is scarce. This being said, the objective of this research was to analyze the effects of dietary ractopamine, and immunocastration, on the quality parameters of an emulsion (frankfurter, Paper 1). From the results of paper 1 it can be concluded that ractopamine did not promote negative effects on frankfurter quality, and also exerted sex-specific effects on the analyzed parameters; products manufactured from surgically castrated barrows exhibit different parameters from those manufactured from gilts and immunocastrated barrows. In order to better understand the molecular base effects of dietary ractopamine on meat quality, we compared the sarcoplasmic proteome of pig loins (*Longissimus thoracis*) from animals fed on ractopamine to their counter-parts (Paper 2). Dietary ractopamine altered the abundance of certain glycolytic enzymes, as well as increased the amount of proteins related to lipogenesis regulation. The increase of lean meat content is due to lipogenesis inhibition; the increase in the protein/fat ratio should be explored by the industry since dietary ractopamine did not adversely affect the quality of emulsion product.

Keywords: Proteome; Pork loin; SDS-PAGE; Immunocastration; Beta-adrenergic agonist

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1 INTRODUCTION

The meat industry constantly seeks for technologies to improve carcass yield, and meat quality to increase revenue and provide high quality products. As opposed to the beef industry, nearly 75% of pork is further processed into meat products; thus, the pork industry requires carcasses with high protein over fat ratio, as protein is widely involved in several technological and sensorial proprieties of meat products. To achieve the desired meat quality, farmers eventually employ feed additives such as ractopamine, an orally administered beta-adrenergic agonist with a repartitioning effect, to increase feed conversion, carcass yield and meat leanness. The mode of action of this drug is associated with the direct activation of beta-adrenergic receptors triggering several biochemical pathways that culminate in the shift of muscle metabolism away from lipogenesis, towards protein anabolism. The veterinary use of this feed additive is approved in few countries such as Brazil, United States, and Canada yet, European Union, China, and Russia have banned its use in livestock.

In addition, other factor affecting pork quality is the boar taint. Surgical castration of male piglets is the standard procedure to prevent this foul smell resulted from the accumulation of androstenone and skatole in meat. The unpleasant odor usually perceived as related to urine and manure is released from pork during cooking. When the animals are entire, their gonads produce and release androstenone, an androgenic hormone; whereas skatole, is produced by enteric bacteria metabolism. The testes removal prevents the androstenone circulation in blood vessels redirecting hepatic enzymes to metabolize more efficiently other molecules such as skatole. The surgical castration stresses the animal due to the invasive procedure and the surgical wound, decreases the animal performance, and potentially increases morbidity and mortality. As an alternative to the surgical castration, there are other methods to control boar taint such as slaughtering the animals before sexual maturity, and immunocastration. The later method promotes testicular atrophy due to autoimmune response against the gonadotropin-releasing factor (GnRF). The immunization is achieved through a two-step vaccination usually at eight and four weeks prior to slaughter. The immunocastration

improves the animal performance due to the presence of the androgenic anabolic hormones during most of animal's life. Furthermore, this method is well accepted by the consumers usually perceived as less violent than the surgical castration. Despite the positive effect of immunocastration over boar taint, animals submitted to this procedure tend to increase the fat deposition, which is considered a negative effect by the meat industry.

Several studies documented the influence of dietary ractopamine on animal performance and fresh meat quality parameters nevertheless, there are few studies examining its effects on processed products. As most pork is further processed into meat products, the investigation of the influence of this feed additive on meat products quality is extremely important. Furthermore, there is lack of information on molecular basis of the effect of dietary ractopamine on meat proteome. Therefore, the objectives of this project were to evaluate the influence of dietary ractopamine and immunocastration on physico-chemical, sensory, and instrumental color and texture qualities of frankfurters; and to investigate the effect of ractopamine feeding on the sarcoplasmic proteome of *Longissimus thoracis*.

2 LITERATURE REVIEW

2.1 PORK INDUSTRY

Pork is the most widely consumed meat in the world, reaching values close to 42% of total consumed meat (PORK CHECKOFF, 2012). Although China accounts for over half of global pork production with over 57 million tons of carcass weight equivalent (CWE), the country exported roughly 275,000 tons in the first months of 2014. European Union was the second largest pork producer (22 million tons CWE), EUA was the third (10 million tons CWE), and Brazil achieved the fourth position with 3.4 million ton CWE. The main importer countries were Japan (1.2 million tons CWE), China and Mexico (0.8 million tons CWE, each); and the main exporters were European Union (2 million tons CWE), Canada (1.3 million tons CWE), and Brazil (0.7 million tons CWE) (USDA, 2014).

Amongst a wide variety of pork products, ham (31.1%), sausages (19.8%), bacon (18.1%), and lunchmeat (10.3%) account for three fourths of the consumed products; fresh cuts account for only 9.2% (PORK CHECKOFF, 2012). This demonstrates that most of the swine carcass is further processed into meat products including ready-to-eat items. Thus, the meat industry has a high demand of meat with high protein to lipid ratio to allow proper water binding, fat holding, and gelation, to provide desirable quality traits (SUN, HOLLEY, 2011). As an example of technology used to improve meat leanness and carcass yield, there is ractopamine, a beta-adrenergic agonist feed additive that promotes protein accretion rather than fat deposition (MERSMANN, 1998).

2.2 SKELETAL MUSCLE PROTEIN TURNOVER

Several proteases are involved in protein metabolism, and can be divided into six groups: cysteine proteases (e.g. calpains, caspases, and most cathepsins), serine proteases (e.g. trypsin), aspartyl proteases (e.g. rennin, pepsin and cathepsin D and E),

glutamic proteases (originally assumed to be limited to fungi (SIMS et al., 2004)), and metalloproteases. In the skeletal muscle tissue, while the animal is alive, proteases play several roles related to enzyme activity, protein turnover, apoptosis, along with others (CRUZEN, 2013). In 1976, Dayton et al. identified calcium activated proteases (calpains) in porcine muscle involved in protein turnover. These proteases are well expressed in skeletal muscle and are referred as μ-calpain (calpain 1) and m-calpain (calpain 2) due to their different calcium concentrations requirement for activation (CONG, GOLL, PETERSON, KAPPRELL, 1989); in addition, there is another calpain (calpain 3) which is specific to skeletal muscle, and requires nanomolar concentration of calcium (GARCÍA DÍAZ et al., 2006). With the calcium binding, the active enzyme cleaves protein sequences at several different points leading to Z-disk hydrolysis. As important substrates there are cytoskeletal proteins such as titin, nebulin, desmin, filamin and synemin (GOLL et al., 2003). In addition, there is an endogenous inhibitor, calpastatin, which interacts with both calpains 1 and 2. Increased calpastatin activity is usually associated with muscle hypertrophy, and decreased proteolysis (GOLL et al., 2003).

During the *post-mortem* period, calpains, cathepsins, caspases and proteasome act on the muscle to meat conversion. At this stage several structural proteins (e.g. desmin, titin, and nebulin), and others such as troponin-T, myosin and actin, are hydrolyzed leading to the Z-disk degradation, meat tenderization and meat aging. Calpastatin plays an important role in the *post-mortem* phase: increased calpastatin activity reflects in decreased protein hydrolysis culminating in a meat with increased toughness (HUFF-LONERGAN, ZHANG, LONERGAN, 2010).

2.3 SKELETAL MUSCLE FIBER TYPE

Different muscle fibers, characterized by their myosin heavy chain (MHC) content, are found in the skeletal muscle tissue. Fiber types I, IIa, IIx and IIb exhibit specific contractile and energetic metabolism; type I fiber is exclusively oxidative, while type II fibers tend to glycolytic metabolism. Amongst type II fibers, IIb is the most glycolytic, IIx is intermediary, and type IIa is oxidative-glycolytic. Oxidative fibers are also known as red fibers and exhibit slow-twitching contraction, while glycolytic ones are refered to as white and fast-twitching fibers (BROOKE, KAISER, 1970). Aside the aforementioned characteristics, type I and II fibers also express distinct metabolic, biochemical and biophysical characteristics such as fiber size, color, and glycogen contents (KARLSSON, KLONT, FERNANDEZ, 1999; KLONT, BROCKS, EIKELENBOOM, 1998). In addition, oxidative muscles contain higher intramuscular fat (IMF) content than glycolytic ones (BROOKE, KAISER, 1970).

Therefore, the fiber type composition of the muscle potentially affects the conversion of muscle to meat and product quality (KARLSSON et al., 1999; KLONT et al., 1998; MALTIN et al., 1997). In pigs, muscles that contain high amounts of fast oxidative fibers (e.g. *Psoas major*) exhibit a more desirable color, higher ultimate pH, greater water holding capacity and better tenderness than glycolytic muscles (*Longissimus dorsi*, and *Semimembranosus* which contain mostly type IIb fibers (CHANG et al., 2003)).

2.4 ACTION OF BETA-ADRENERGIC AGONISTS

Beta-adrenergic agonists bind to β-receptors and act similarly to endogenous catecholamines (epinephrine, norepinephrine). The cellular response to this type of molecule is related to the receptor subtype and its abundance on the cell membrane that upon activation, triggers several biochemical cascades through phosphorylation, culminating in increased lean meat in livestock (KUTZLER et al., 2011).

The increase on the muscle mass is achieved due to increased protein synthesis (myosin light chain, α-actin, and calpastatin) via cAMP/protein kinase A signaling promoting protein transcription (LYNCH, RYALL, 2008). In addition, the stimulation of β-receptors promotes increase on calpastatin and m-calpain activities decreasing net protein turnover (BARDSLEY et al., 1992). The increase on m-calpain activity is potentially associated with the increased skeletal muscle growth rate, allowing some proteins to be hydrolyzed to increase substrate for further protein synthesis (GOLL et al., 2003). Beta-adrenergic agonists also promote muscle fiber type shift from oxidative phenotype towards glycolytic one, following the regular transition pathway: I → IIa → IIx → IIb (DEPREUX et al., 2002). Furthermore, beta-adrenergic agonists regulate lipid turnover via fatty acid synthesis and β-oxidation promoting tri-, di-, and mono-acylglycerol hydrolysis, and release of free fatty acids. The activation of β-receptors in adipocytes promotes lipolysis which is also associated with decrease in lipogenesis (HALSEY et al., 2011).

Ractopamine has been popularly used as a feed additive to improve livestock performance for several years (BOLER et al., 2011). Its molecular structure is depicted in Figure 1.

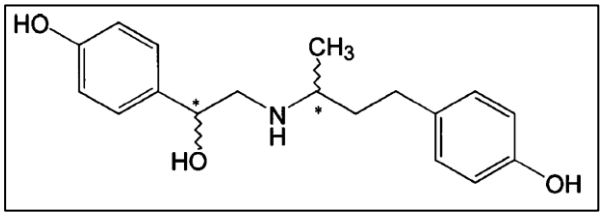

Figure 1. Ractopamine structure (RICKE et al., 1999)

The asterisks indicate the two chiral carbons in ractopamine structure demonstrating that the molecule exists as a mixture of four stereoisomers (RICKE et al., 1999), each promoting distinct β-receptors activation (MILLS, SPURLOCK, SMITH, 2003). Data from previous research indicate that ractopamine binds to both β1 and β2 receptors in swine adipocytes (MILLS et al., 2003). In addition, the growth genotype also

affects the dietary ractopamine efficiency in finishing pigs; slower to moderate growing genotypes exhibit greater improvement than faster genotypes (MILLS, et al., 1990).

2.5 EFFECT OF DIETARY RACTOPAMINE ON CARCASS AND MEAT QUALITY

Several authors reported the influence of dietary ractopamine on carcass and meat qualities in finishing pigs (for details refer to ANDRETTA et al., 2012, APPLE et al., 2007 and BOHRER et al., 2013). Briefly, carcasses from animals fed on ractopamine exhibit greater hot carcass weight (RINCKER et al., 2009), heavier whole ham (CARR et al., 2009; KUTZLER et al., 2011), increased loin eye area (CARR et al., 2009) than carcasses from animals fed on control diets. In addition, based on the meta-analysis documented by Apple et al. (2007) dietary ractopamine promotes no detrimental effects on fresh pork color, firmness, water-holding capacity, marbling, or intramuscular fat. Muscle pH is an important parameter routinely measured to estimate the overall meat quality, as pH affects color, firmness and water holding capacity. Studies have documented that dietary ractopamine (5 mg/kg) did not alter the swine longissimus ultimate pH (PATIENCE et al., 2009). In terms of sensory quality, it is a controversial topic because there is no agreement amongst the available literature. Some authors reported increased shear force (PATIENCE et al., 2009) in pork obtained from animals fed on ractopamine while others, documented no difference (KUTZLER et al., 2011).

2.5.1 Ractopamine residue in meat

Consumers are continuously concerned about the health impact of veterinary drugs, food additives and ingredients. Human food poisoning caused by beta-adrenergic agonists was already documented (PULCE et al., 1991) however, it was related to the consumption of liver from clembuterol-fed veal; a highly potent drug that is not commonly used in pig production. In terms of ractopamine residue, it was reported that

the actual orally fed molecule is the main residue accumulated in swine skeletal muscle tissue (QIANG et al., 2007) and its concentration were not detectable or below 10 µg/kg (PLEADIN et al., 2012; FAO, 2010) at 24 h after last day of treatment with a diet containing up to 27.9 mg/kg of ractopamine. In addition, adipose tissue exhibits similar values to those observed in skeletal muscle. Based on several reports, the acceptable daily intake are 1.25 µg/kg (FDA, 2013), and 0—1 µg/kg (FAO, 2010) of person body weight per day, and the maximum recommended limit are 10 µg/kg (FAO, 2010) and 30 µg/kg (FDA, 2013) for the swine meat.

2.6 BOAR TAINT

The undesirable foul smell in boar meat is caused by the accumulation of a testicular steroid, identified as 5 α-androst-16-en-3-one (androstenone; urine-like sex odor), and 3-methyl-indole (skatole; fecal odor), in the adipose tissue. Although androstenone and dihydrotestosterone (5 α-dihydrotestosterone) are both synthetized in the Leydig cells of the testicles, these molecules exhibit different biological functions: the former one is a sex pheromone, while dihydrotestosterone is an anabolic steroid. Due to the high lipophilic characteristic of androstenone, when accumulated in the fat tissue, it requires 3—6 weeks to be cleared from the animal organism (CLAUS, WEILER, HERZOG, 1994).

Skatole, the other molecule involved in boar taint, is produced by intestinal bacteria from the degradation of tryptophan. The biochemistry (Figure 2) involved in skatole formation starts with deamination of tryptophan by unspecific bacteria, favoring the formation of indole acetic acid (IAA), followed by decarboxylation of IAA into skatole by specific bacteria (WHITEHEAD et al., 2008). Furthermore, as skatole formation is not related to sex-specific organs, its accumulation in the adipose tissue is not limited to boar. However, as the testicles continue to secrete steroids in boars, hepatic enzymes are directed to metabolize these hormones disfavoring the breakdown of other substances such as skatole (EFSA, 2004).

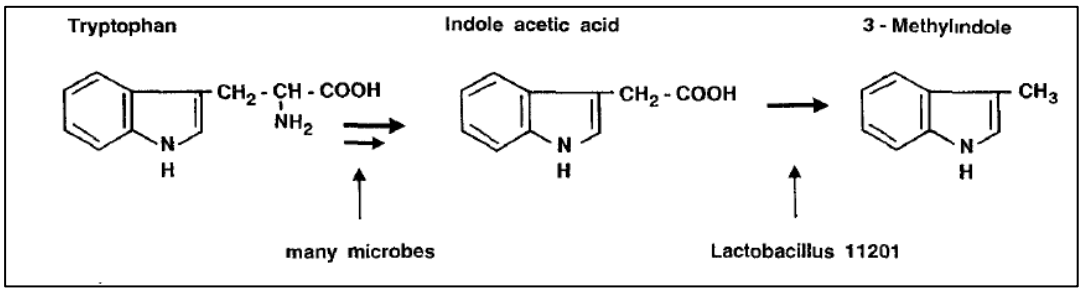

Figure 2. Tryptophan metabolism by hindgut flora (CLAUS et al., 1994)

The traditional method for controlling boar taint is the surgical castration of male piglets at 7—10 days of age; generally *gubernaculum testis* and spermatic cords are lacerated without the use of analgesia and anesthesia (EFSA, 2004). This procedure has several drawbacks such as decrease on growth rate due to increased morbidity, and increased risk of mortality. Furthermore, the increasing concerns about animal welfare, favors less invasive and more humane animal handling and procedures (VANHONACKER, VERBEKE, 2011; HEID, HAMM, 2013; EFSA, 2004). Consequently, alternative methods to surgical castration such as slaughter of boar before pubertal development, and immunocastration receive more attention.

2.7 IMMUNOCASTRATION

The testes are regulated by hormones produced by the pituitary gland, namely follicle stimulating hormone (FSH) and luteinizing hormone (LH). Under normal circumstances, the hormonal cascade can be summarized as: the hypothalamus secretes GnRF that binds to specific receptors at the pituitary gland, and stimulates the release of the gonadotropins FSH and LH. The gonadotropins will in turn stimulate the testes (Sertoli and Leydig cells) promoting sperm maturation and steroids secretion. The immunocastration method is based on the vaccination of boars with injection containing a GnRF analogous that does not bind to the specific receptors (does not trigger pituitary secretion of FSH and LH), and stimulates autoimmune response against GnRF. The

immunization protocol for pigs is composed by two injections: the first dose primes the immune memory cells; while the second promotes the increase on the specific anti-GnRF antibodies concentration. These antibodies bind to the circulating GnRF and neutralize it. The first shot is usually applied eight weeks prior to slaughter, while the second, four weeks. After the second dose of the immunocastration vaccine, the temporary decrease on the pituitary gland stimulation inhibits testicular function due to the lack of gonadotropins. In addition, the testes tend to atrophy ceasing the production and release of testosterone and androstenone (HENNESSY, 2008).

The immunocastration improves the animal efficiency as boar exhibits better animal performance (heavier carcasses and leaner meat) than barrow. Immunocastrated barrows tend to exhibit better animal performance (average daily gain, average daily feed intake, and feed conversion) than surgically castrated ones (DUNSHEA et al., 2001). In terms of carcass quality, immunocastrates exhibit leaner and more muscular carcasses with greater lean meat yields than their surgically castrated counter-part. As for meat quality, immunocastration does not negatively influence longissimus ultimate pH (PAULY et al., 2009; BOLER et al., 2012) nor its shear force at 14 d postmortem (BOLER et al., 2012).

There is limited information available on the influence of dietary ractopamine on immunocastrates meat quality and further meat processing. Still, the two technologies potentially act synergistically improving, or not negatively affecting, growth performance, carcass quality and pork qualities such as pH, color and tenderness (LOWE, 2013; MOORE et al., 2009). Although fresh meat quality has been widely studied, information regarding the effects of such technologies on the sarcoplasmic proteome of pork is limited.

2.7.1 Residue of immunocastration treatment in meat

The Food and Drug Administration conducted a worst-case residue exposure evaluation for total drug residue in edible tissues, including the injection site and concluded that the consumption of meat from immunocastrates represents no risk for the human health (FDA, 2011).

2.8 PROCESSED MEAT PRODUCT QUALITY—FRANKFURTER

Frankfurters are ready-to-eat cooked meat emulsions mainly composed by lean meat, fat, starch, seasonings, water, and salt; therefore, the ability of the meat batter to form a stable matrix holding water, lipids and other ingredient, is extremely important (COLMENERO, 1996). Furthermore, the color in comminuted products is governed by fat, moisture contents (AHMED et al., 1990), and myoglobin content and its chemical state within the meat used to manufacture the product (HAND et al., 1987). In terms of texture, low fat sausages exhibit greater hardness than high fat ones (CENGIZ, GOKOGLU, 2007; TOBIN et al., 2013), due to increased protein content that in turn favors a more stable matrix (COLMENERO, 1996). The consumer purchase decision was previously related to taste (CHAMBERS, CHAMBERS, BOWERS, 2007) and packaging visual aspects (GONZÁLEZ-VIÑAS et al., 2004) notwithstanding, odor, texture and appearance of the product are also considered during product appraisal (MEILGAARD, CIVILLE, CARR, 2007). Thus, it is important to investigate the influence of dietary ractopamine and immunocastration on the meat product quality.

2.9 DIETARY RACTOPAMINE AND IMMUNOCASTRATION—MEAT PRODUCT

Although most of pork if further processed into meat products (PORK CHECKOFF, 2012), to the best of our knowledge, there is limited information regarding

the effects of dietary ractopamine, and immunocastration, individually or combined, on further processed products quality. Tavárez et al. (2012) investigated the quality of pork shoulder from ractopamine-fed pigs and observed no detrimental effect on processing and sensory characteristics. In another study, Boler et al. (2011) evaluated the influence of dietary ractopamine on the quality of cured ham and its processing. The authors documented that cooking yield values were not different between products from ractopamine-fed pig and their control counterparts; however, cured hams from the ractopamine group exhibited greater *L** (lightness) and lower *a** (redness) values than control. Moreover, immunocastration did not negatively influence the quality parameters of dry-cured hams (FONT I FURNOLS et al., 2012). As both technologies affect raw meat protein quality and concentration, further studies investigating if such changes influence meat product processing and final product quality are necessary.

2.10 PROTEOMIC ANALYSIS AND MEAT QUALITY

Meat quality traits are governed by exogenous (environment and processing) and endogenous (genetic) factors. Studies investigating the genomic field have proposed several genetic biomarkers for meat quality. However, the desired phenotype is dynamically governed by mRNA translation, and protein synthesis, modification, interaction and complexation; the solely presence of a gene biomarkers does not mean that its trait will be expressed. While the genome holds all the information available from certain cell, the proteome contains the information from genes that are being expressed given a specific condition (TYERS, MANN, 2003).

Several studies employed gel-based analysis to investigate the influence of gender, breed (HOLLUNG et al., 2009) and others (KWASIBORSKI et al., 2008a), on the swine proteome, and its relation to pork quality (KWASIBORSKI et al., 2008b), meat aging (DI LUCA et al., 2011; 2013), myoglobin oxidation (SUMAN et al., 2006) and the conversion of muscle to meat (LAMETSCH, BENDIXEN, 2001; LAMETSCH et al., 2003; HWANG et al., 2005), In addition, Picariello et al. (2006) used two electrophoretic

approaches coupled with tandem mass spectrometry analysis, to separate and investigate the sarcoplasmic proteome of the swine ham, as well as the proteomic differences between skeletal and cardiac muscle in pigs were investigated by Hornshoj et al. (2009). The aforementioned studies identified several proteins involved in energy metabolism, and proteolysis, and suggested that they are potential biomarkers for meat quality. Nonetheless, there is limited information on molecular basis investigating the influence of dietary ractopamine on the sarcoplasmic proteome of pork loin.

3 MANUSCRIPTS

3.1 CHAPTER 1: SEX-SPECIFIC EFFECT OF RACTOPAMINE ON QUALITY ATTRIBUTES OF PORK FRANKFURTERS. Published in *Meat Science*, v. 96, p. 799—805, 2014 (Paper 1)

Bruno R.C. Costa-Lima [a,*], Anna C.V.C.S. Canto [a], Surendranath P. Suman [b], Carlos A. Conte-Junior [a], Expedito T.F. Silveira [c], Teofilo J.P. Silva [a]

[a] Departamento de Tecnologia de Alimentos, Faculdade de Veterinaria, Universidade Federal Fluminense (UFF), Niteroi 24230-340, Rio de Janeiro, Brazil

[b] Department of Animal and Food Sciences, University of Kentucky, Lexington, KY 40546, USA

[c] Centro de Tecnologia de Carnes, Instituto de Tecnologia de Alimentos, Campinas 13070-178, Sao Paulo, Brazil

* Corresponding author. Tel.:+55 21 2629-9545; E-mail address: brunuff@gmail.com (B.R.C. Costa-Lima).

3.1.1 Abstract

Our objective was to determine the effect of dietary ractopamine and immunocastration on the quality attributes of pork frankfurters. Gilts (GT), surgically castrated male pigs (SR) and immunologically castrated male pigs (IM) were fed diets containing 7.5 ppm ractopamine (RAC) or no ractopamine (CON) for 21 days prior to harvest. Deboned hams were manufactured into frankfurters, and physico-chemical parameters, instrumental color and texture, and sensory attributes were evaluated. Ractopamine increased ($P < 0.05$) L^* (lightness) in SR, whereas it decreased ($P < 0.05$) lightness in IM and GT. While ractopamine increased ($P < 0.05$) a* (redness) in GT, a reverse ($P < 0.05$) trend was observed in SR. With respect to instrumental texture, ractopamine increased ($P < 0.05$) hardness, resistance, and springiness in IM, cohesiveness and springiness in GT, and shear force in SR. These results indicated that ractopamine exerted sex-specific effects on frankfurter quality. Swine industry may adopt sex-specific dietary strategies to optimize the quality of further processed meat products.

Keyword: Ractopamine; Immunocastration; Pork frankfurter; Instrumental quality; Sensory quality

3.1.2 Introduction

Boar taint is a major problem in pork industry caused by androstenone produced by testes (Patterson, 1968) and skatole generated by intestinal bacteria (Claus, Weiler, & Herzog, 1994). Surgical castration of piglets is employed to control boar taint (Prunier et al., 2006). However, this strategy decreases growth performance as it stops production of androgenic hormones. An alternative to surgical castration is immunocastration, which is achieved by immunizing male pigs against gonadotropin releasing factor (GnRF). Immunocastration decreases production of testicular hormones and minimizes accumulation of androstenone (Zamaratskaia et al., 2008). The decrease in testicular hormone enhances hepatic metabolism of skatole and decreases its accumulation (Doran, Whittington, Wood, & McGivan, 2002; Lin, Lou, & Squires, 2004a, 2004b). This approach allows entire male pigs to attain increased body weight (favored by testicular hormones), while preventing boar taint (Dunshea et al., 2001; Jaros et al., 2005). Immunocastration has been approved in swine production in more than 50 countries (Gispert et al., 2010). Previous investigations reported that immunocastrated male pigs exhibited meat quality attributes (instrumental and sensory) comparable to barrows and gilts (Boler et al., 2012; D'Souza & Mullan, 2002; Font i Furnols et al., 2008, 2009; Pauly, Luginbuhl, Ampuero, & Bee, 2012).

Ractopamine is a phenethanolamine repartitioning agent with beta-adrenergic agonist properties and is used to promote leanness in meat animals. The species-specific effects of ractopamine on protein metabolism and lipogenesis are well documented in meat-producing livestock (Avendano-Reyes et al., 2006; Dunshea, D'Souza, Pethick, Harper, & Warner, 2005; Liu & Mills, 1989; Lopez-Carlos et al., 2011). The effects of ractopamine on pork quality are associated with increased myofibrillar protein synthesis and improved carcass yield, cutability, and leanness (Adeola, Ball, & Young, 1992; Bohrer et al., 2012; Carr et al., 2009; Kutzler et al., 2011). Dietary ractopamine offers an excellent strategy to maximize the positive effects of

immunocastration while controlling its negative effects such as increase in fat mass (Lanferdini et al., 2013). Therefore, a combination of immunocastration and ractopamine feeding has the potential to improve meat quality in pigs (Rikard-Bell et al., 2009).

In the modern-day meat industry, more than three-fourths of pork is further processed and marketed as ready-to-eat products such as ham, bacon, and sausage (Pork Checkoff, 2012). Thus it is important to determine the effect of ractopamine and immunocastration on further processed pork products. While several previous investigations focused on fresh pork quality, limited number of studies examined the effect of ractopamine and immunocastration on further processed pork products. Boler et al. (2011) and Font i Furnols et al. (2012) concluded that immunocastration does not necessarily influence quality parameters of hams. However, investigations are yet to be undertaken to evaluate the impact of ractopamine feeding and immunocastration on emulsion-type pork products. Frankfurters are pre-cooked emulsion-type sausages having large market share. They are popular fast-food items and are widely consumed. Therefore, the aim of the present study was to evaluate the influence of dietary ractopamine on the sensory and instrumental attributes of pork frankfurters processed from immunocastrated male pigs, surgically castrated male pigs, and gilts.

3.1.3 Materials and methods

3.1.3.1 Animal production

The research protocols were in accordance with the guidelines of the Institutional Ethics Committee on Animal Use and the Ministry of Agriculture, Brazil. Ten female and twenty male piglets (AGPIC 337 male × CB22 female, Agroceres PIC, Sao Paulo, Brazil) were used in this study. All the animals were raised under similar conditions at a commercial swine production facility at Sao Paulo, Brazil. The piglets were selected when they were 5 days old and had a body weight of 1.5 kg. The sexes of the animals used in this study were gilts (GT), immunocastrated male pigs (IM), and surgically castrated male pigs (SR). Ten animals per sex were allocated. The animals were

weaned at three weeks and were group-penned based on sex until 21 days prior to the harvest. Immunocastration was accomplished by vaccinating male pigs against GnRF with VIVAX (200 µg of GnRF–protein conjugate per ml of an aqueous adjuvant system; Pfizer, Brazil) at eight weeks and four weeks prior to slaughter. Surgical castration was performed when male piglets were seven days old.

The animals were finished for 175 days on a commercial diet to reach an average body weight of 115 kg. Twenty-one days before the harvest, five animals (n = 5) from each sex were randomly selected and fed a diet containing either 0 ppm (CON) or 7.5 ppm ractopamine (RAC; Paylean, Elanco Animal Health, Greenfield, IN, USA) until harvest. During those 21 days, the animals were group-penned based on six treatments (3 sexes × 2 diets). Thus the six treatments in this study were GT-CON, GT-RAC, IM-CON, IM-RAC, SR-CON, and SR-RAC. The animals were humanely harvested in a packing plant (Sao Paulo, Brazil) under Brazilian federal inspection. The carcasses were chilled at 2 °C for 24 h and were fabricated. The green hams (average weight of 9.8 kg) from the left side of the carcasses were deboned, and meat and fat were separated. The meat and fat from each deboned hams were separately bulk-packaged in polyvinyl chloride film, frozen at −18 °C, and transported to the pilot plant of the Instituto de Tecnologia de Alimentos (Campinas, Sao Paulo, Brazil) for further processing.

3.1.3.2 Frankfurter processing

Upon arrival at the Instituto de Tecnologia de Alimentos, each deboned ham was separately processed into frankfurters on the same day to provide five replicates (n=5) per treatment. Frozen meat and fat were chopped to small pieces and were separately ground using a 5 mm plate. One 8-kg batch of emulsion was prepared from each deboned ham based on the formulation presented in Table 1. Meat was transferred to a bowl chopper, and salt was added. Meat was comminuted for 3 min at low speed to extract myofibrillar proteins until the temperature reached 6°C, when other ingredients were slowly added. The temperature of the mixture was not allowed to exceed 12°C. The emulsion was stuffed in cellulose casings (29 mm diameter) forming 100g links. The frankfurter strings were placed in an industrial oven at 55°C for 40min for drying, and the

temperature was increased 5 °C every 10min until the oven temperature reached 80 °C. The frankfurters were cooked to an internal temperature of 75 °C. The internal temperature was monitored by thermocouples. The cooked frankfurters were cooled using a water shower (7 °C) for 10min. The casings were peeled off manually using knife, and the frankfurters were vacuum packaged. The vacuum packaged frankfurters were heat-treated at 80°C for 5s, in a water bath, to decrease post-cooking bacterial contamination and were then stored at 4 °C until further analysis.

3.1.3.3 Water activity, pH, and proximate composition.

Frozen meat and cooked frankfurters were analyzed for pH, water activity (aw), and proximate composition. The pH was measured using a probe pH meter (Model DM-21, Digimed, Sao Paulo, Brazil), whereas a_w was determined at 25°C using Aqualab Decagon CX-2T water activity meter (Decagon, Pullman, WA, USA). The moisture, protein, and lipid contents were determined according to AOAC (2005).

3.1.3.4 Cooking yield

The initial weight of raw frankfurter strings was recorded. After cooking and cooling, the strings were blotted, and the final weight was measured. Cooking yield was calculated from differences in the initial and final weight and expressed as percentage of initial weight (Boles & Swan, 1996).

3.1.3.5 Instrumental color

Cooked frankfurters were bisected parallel to their long axis and maintained at 25°C for 30min before color evaluation. CIE *L** (lightness), *a** (redness), *b** (yellowness), hue, and chroma values were measured at two random locations on each sample using a Minolta CM-508d spectrophotometer (Osaka, Japan) with 8 mm diameter aperture, illuminant D65, and 10° standard observer (AMSA, 2012).

3.1.3.6 Instrumental texture

Shear force and texture profile (Bourne, 1978) of frankfurters were evaluated using TA-TX2i texture analyzer (StableMicro System, Surrey, United Kingdom). Samples were cut into 2.5 cm length cylinders and held at 25 °C. For measuring shear force, Warner–Bratzler blade with a triangular notch was used. The samples were placed on the guillotine block and sheared completely as the blade moved down at the speed of 5 mm/s. For texture profile, a cylindrical metal probe of 35 mm diameter was used. The samples were compressed to 70% of their original height in two cycles at pre-test speed of 5 mm/s, test speed of 1 mm/s, and post-test speed of 5 mm/s. The time between the compressions was 2 s. The data on texture profile were obtained and processed by Texture Expert Software (Stable Micro System, Surrey, United Kingdom) and expressed as hardness, springiness, cohesiveness, and resistance.

3.1.3.7 Sensory evaluation

Consumer acceptance test was conducted to evaluate appearance, odor, texture, flavor, overall acceptance, and purchase intention. Forty-nine untrained panelists (21–30years old) among students, faculty, and staff of the Instituto de Tecnologia de Alimentos were recruited. The panelists were regular consumers of frankfurters. The individual booths were equipped with computers. The coded samples were presented to panelists in randomized blocks and in a sequential monadic way. For each treatment, two 2-cm cuts of frankfurters were provided to the panelists. A nine-point hedonic scale (1 = dislike extremely; 2 = dislike very much; 3 = dislike moderately; 4 = dislike slightly; 5 = neither dislike nor like; 6 = like slightly; 7 = like moderately; 8 = like very much; 9 = like extremely) was used for appearance, odor, texture, flavor, and overall acceptance, whereas purchase intention was evaluated on a five-point scale (1 = certainly would not buy; 2 = probably would not buy; 3 = may or may not buy; 4 = probably would buy; 5 = certainly would buy). Drinking water and unsalted crackers were offered for cleaning the palate between samples (Stone & Sidel, 1993). Data were collected using the computers equipped with Compusense Five Version 4.2 software (Compusense Inc., Guelph, Ontario, Canada).

3.1.3.8 Statistical analysis

The experiment was a completely randomized design. The treatments were arranged as a full factorial of 3 sexes and 2 ractopamine levels. All the traits were analyzed using a linear model, which considered sex, ractopamine level, and their interactions as fixed effects. Each ham was considered as an experimental unit, and each one of the six treatments (3 sexes × 2 ractopamine levels) had five hams. Each deboned ham was individually processed into frankfurter to provide five replicates (n=5) per treatment. Data for physico-chemical attributes, instrumental color, and instrumental texture were analyzed using the MIXED procedure (SAS, 2009). When the analysis of variance detected differences among the treatments at a significance level of 5%, the Tukey–Kramer test was applied to discriminate the least squares means. Sensory data were analyzed using ANOVA in Compusense Five Version 4.2 software, and the differences among means were detected at 5% level of significance using Tukey's HSD test.

Additionally, the data (physico-chemical attributes, instrumental color, instrumental texture, and sensory) were evaluated by principal component analysis in a correlation matrix using XLStat software (Addinsoft, Paris, France). In the correlation matrix, the data were centered and scaled based on the parameter means. The matrix consisted of 21 columns and 6 rows, with the columns representing the means of parameters and the rows representing treatments. For the sensory attributes, the scores from all the 49 panelists were averaged for each treatment to calculate the means. Parameter means demonstrating square cosines greater than 0.60 were considered for principal components; values close to 1 indicate that the parameter is well projected on the axes (Le, Josse, & Husson, 2008).

3.1.4 Results and discussion

3.1.4.1 Physico-chemical attributes of meat from deboned hams

The data on physico-chemical attributes of meat from fresh deboned hams are presented in Table 2. There were interactions ($P < 0.05$) between sex and dietary ractopamine on all the attributes; the results indicate that the effect of ractopamine is sex-specific.

Dietary ractopamine increased ($P < 0.05$) the pH of meat from GT and IM, but not in SR (PN 0.05). SR-RAC, SR-CON, and GT-RAC exhibited the greatest ($P > 0.05$) pH values followed by GT-CON and IM-RAC; IM-CON had the lowest ($P < 0.05$) pH value. Our findings on pH are in disagreement with those reported by Font i Furnols et al. (2012), who observed that semimembranosus muscle from immunocastrated Duroc barrows had greater pH than those from gilts. The differences in pH between the present study and Font i Furnols et al. (2012) can be attributed to the differences in the breed and muscle source (Monin, Mejenes-Quijano, Talmant, & Sellier, 1987).

Ractopamine did not influence ($P > 0.05$) water activity of meat. However, within CON diet groups, SR demonstrated greater ($P < 0.05$) water activity than GT. There were no differences ($P > 0.05$) between the groups within RAC diet indicating that dietary ractopamine nullified the variations in water activity. Ractopamine supplementation increased ($P < 0.05$) the moisture content of meat only in IM, whereas it increased ($P < 0.05$) the protein content in SR and IM. Lipid content of meat was decreased ($P < 0.05$) by ractopamine in GT and IM.

It is well documented that ractopamine increases leanness in finishing pigs (Andretta et al., 2012; Boler et al., 2010; Carr et al., 2009; Kutzler et al., 2011; Rikard-Bell et al., 2009). The mode of action of beta-agonists is through modulation of calpain-dependent protein turnover (McDonagh, Fernandez, & Oddy, 1999; Wheeler & Koohmaraie, 1992) by increasing calpastatin expression. This is reflected in the greater protein content in meat from IM-RAC and SR-RAC than their CON counterparts. The high protein content in meat increases alkaline components, which can result in an increase in pH value. This could have partially contributed to the greater pH of IM-RAC than their control counterparts. In contrast to our results, several authors reported no difference in the ultimate meat pH between GT, SR, IM, and entire boars (Boler et al.,

2010; Gispert et al., 2010; Pauly, Spring, O'Doherty, Kragten, & Bee, 2009; Zamaratskaia et al., 2008).

Ractopamine demonstrated a pronounced effect on IM animals; meat from these animals contained greater protein and moisture and lower lipid contents than their control counterparts. These results indicated that the negative effects of immunocastration, such as low moisture and high lipid contents can be mitigated by dietary ractopamine. While ractopamine decreased the lipid contents in meat from the gilts, this effect was not observed in SR. Our results suggest that ractopamine is not effective in SR to improve meat proximate composition. Although ractopamine is a potent stimulator of adipose tissue lipid mobilization, it often does not reduce fat deposition because of rapid down-regulation of beta-adrenergic receptors, lack of effect on lipogenesis, and/or reduced sensitivity to beta-agonists (Rikard-Bell et al., 2009).

Our data are in agreement with previous research, which reported that ractopamine favors protein accretion (Carr et al., 2009; Dunshea, King, Campbell, Sainz, & Kim, 1993; Rikard-Bell et al., 2009). The effects of sex observed in the present study are in agreement with Carr et al. (2009), who reported that gilts had greater protein content in soft tissues than surgically castrated males. In contrast, Boler et al. (2010) did not observe any differences in the protein content of fresh hams from gilts and barrows.

3.1.4.2 Physico-chemical attributes of frankfurters

There was no interaction ($P > 0.05$) between sex and dietary ractopamine on cooking yield, pH, and protein content. While sex influenced ($P < 0.05$) cooking yield and pH (Table 3), ractopamine influenced ($P < 0.05$) pH and protein content (Table 4). Frankfurters from IM exhibited greater ($P < 0.05$) cooking yield than those from other sexes. SR demonstrated greater ($P < 0.05$) pH than IM. Ractopamine increased ($P < 0.05$) both pH and protein content in frankfurters.

The data on frankfurter parameters influenced by the interaction ($P < 0.05$) between dietary ractopamine and sex are presented in Table 5. There was no effect ($P > 0.05$) of RAC on water activity. Although SR-CON exhibited lower ($P < 0.05$) water activity than GT-CON, their RAC counterparts had similar ($P > 0.05$) values. GT-RAC

demonstrated the greatest ($P < 0.05$) water activity, while SR-CON had the lowest ($P < 0.05$). Dietary ractopamine increased ($P < 0.05$) moisture content of frankfurters from GT and IM. Frankfurters from IM-RAC and GT-RAC showed greater ($P < 0.05$) moisture contents than other groups; GT-CON had the lowest ($P < 0.05$) moisture content. Frankfurters manufactured from ractopamine fed GT and IM demonstrated lower ($P < 0.05$) lipid content than their control counterparts reflecting their decreased lipid contents in meat (Table 2).

Emulsion-type meat products are a complex matrix, wherein water droplets, fat globules, and other ingredients are entrapped within a protein network. The variations in lipid, moisture, and protein content in fresh meat, due to sex and diet, can thus influence the quality of such products. To our knowledge, the present study is the first to evaluate the influence of immunocastration and dietary ractopamine on the quality attributes of emulsion-type pork products. The previous investigation on hams (Boler et al., 2010) reported no effect of sex, ractopamine feeding, or their interaction on cooking yield. Our data are in partial agreement with aforementioned authors as GT and SR as well as RAC and CON had similar results for cooking yield.

3.1.4.3 Instrumental color of frankfurters

Color parameters exhibited sex-specific response ($P < 0.05$) to RAC (Table 6). Dietary ractopamine decreased L^* values ($P < 0.05$; promoted darker appearance) in frankfurters from GT and IM, while it increased L^* values ($P < 0.05$; promoted lighter appearance) in SR. Ractopamine increased ($P < 0.05$) a^* values (redness) in GT, while it decreased ($P < 0.05$) redness in SR. IM-CON and GT-RAC exhibited greater ($P < 0.05$) a^* values than GT-CON and SR-RAC. The b^* values (yellowness) of frankfurters from ractopamine fed IM were greater ($P < 0.05$) than their counterparts in control diet. In addition, IM-RAC exhibited the greatest (P b< 0.05) b^* value, whereas IM-CON had the lowest ($P < 0.05$) b^* value. While ractopamine increased ($P < 0.05$) chroma in IM, the reverse ($P < 0.05$) was observed in SR. On the other hand, RAC increased ($P < 0.05$) the hue values in SR and IM.

The range of data for color parameters in the present study is in agreement with those of Choe, Kim, Lee, Kim, and Kim (2013) in pork frankfurters. Pietrasik (1999) as well as Cengiz and Gokoglu (2007) reported that the color of frankfurter-type products is influenced by fat content, added water, and pigmentation of the raw meat used. Increased lipid content can decrease redness; this was observed in frankfurters manufactured from gilts, where GT-CON demonstrated lower *a** and greater lipid content than GT-RAC (Table 5). Non-meat ingredients are added to increase extractability of meat proteins and for improving emulsion stability. Furthermore, cooking-induced protein denaturation influences physico-chemical properties of emulsion (Totosaus, Montejano, Salazar, & Guerrero, 2002). Together, these changes could have modulated some of the observed effects of ractopamine and immunocastration on pork frankfurters.

3.1.4.4 Instrumental texture of frankfurters

The instrumental texture parameters of frankfurters are presented in Table 7. There was interaction ($P < 0.05$) between sex and diet. Dietary ractopamine increased ($P > 0.05$) hardness of frankfurters manufactured from IM. While IM-RAC demonstrated greater ($P < 0.05$) hardness than GT-RAC, SR-CON exhibited greater ($P < 0.05$) hardness than IM-CON. With respect to springiness, frankfurters from GT-RAC and IM-RAC animals exhibited greater ($P < 0.05$) values than their control counterparts. Frankfurters from GT-CON demonstrated greater ($P < 0.05$) cohesiveness than GT-RAC. Ractopamine increased ($P < 0.05$) resistance of frankfurters from IM. RAC did not influence ($P > 0.05$) hardness, springiness, cohesiveness, and resistance of SR (Table 7). The effect of dietary ractopamine on frankfurter shear force was sex-specific ($P < 0.05$; Table 7). While RAC increased ($P < 0.05$) shear force in GT, the reverse effect ($P < 0.05$) was observed in SR. There was no effect ($P > 0.05$) of RAC on shear force in IM. SR-CON demonstrated the greatest ($P < 0.05$) shear force, whereas IM-CON, IM-RAC, and GT-CON had the lowest ($P < 0.05$) values.

Although the present study was the first to report differences in textural attributes of frankfurters from ractopamine fed pigs, previous investigations (Athayde et al., 2012;

Xiong et al., 2006) observed an increase in the shear force values of whole-muscle pork loins from ractopamine fed animals. It is not appropriate to extrapolate the observations from these studies to frankfurters because while manufacturing emulsions the meat is finely chopped and the muscle structure is disrupted.

3.1.4.5 Sensory evaluation

There was no interaction ($P > 0.05$) between sex and dietary ractopamine on the sensory attributes. The effect of ractopamine on sensory attributes is presented in Table 8; RAC frankfurters exhibited better ($P < 0.05$) texture than their CON counterparts. The improved texture for RAC frankfurters can be partially explained on the basis of their relatively high moisture and protein contents (Tables 4 and 5).

The effect of sex on sensory attributes is presented in Table 9. Flavor and purchase intention were the only parameters influenced ($P < 0.05$) by sex. Frankfurters from SR had greater ($P < 0.05$) flavor scores than GT and IM. This could have contributed, in part, to the greater ($P < 0.05$) purchase intention for frankfurters from SR than those from IM. On the other hand, the panelists rated the frankfurters from three sexes similar ($P > 0.05$) in appearance, odor, and texture. Appearance is the first attribute considered by consumers in appraising product quality, followed by odor, texture, and then flavor (Meilgaard, Civille, & Carr, 2007). Based on this stepwise approach, the sensory data suggest that frankfurters from different sexes are rated similar up to the texture. The last perceived attribute (flavor) was the one that differentiated the treatments; SR received greater flavor scores than the other sexes, which could be partially explained based on the relatively high lipid content in SR frankfurters (Table 5).

3.1.4.6 Principal component analysis of frankfurter quality parameters

This multivariate analysis explained 77.43% of total variance in data (Fig. 1). Principal component 1 contributed to 47.10% of the variance and categorized the treatments into two groups; the first group consisted of GT-CON and IM-CON, whereas

the second group was formed by SR-CON, SR-RAC, IM-RAC, and GT-RAC. This categorization was based on appearance, flavor, odor, texture, purchase intention, pH, hue, and chroma. Several parameters demonstrated strong correlation with others. The sensory texture was positively correlated to pH (r = 0.925) indicating that pH plays an important role in texture. Furthermore, purchase intention was positively correlated to appearance (r = 0.972), flavor (r = 0.985), and sensory texture (r = 0.891) scores, indicating the criticality of these parameters in consumers' purchase decisions. The second group (consisting of SR-CON, SR-RAC, IM-RAC, and GT-RAC) had greater sensory scores than the first group.

Principal component 2 explained the 30.33% of the total variance and categorized the treatments into two different groups, one consisting of SR-CON, GT-RAC and IM-RAC; and another one comprising SR-RAC, GT-CON and IM-CON. The parameters related to principal component 2 were *a** value, springiness, and moisture and lipid contents. While lipid content was negatively correlated to moisture content (r = −0.977) and springiness (r = −0.955), moisture content was positively correlated to springiness (r = 0.879). Frankfurters manufactured from SR-CON, GT-RAC and IM-RAC were rated better in springiness than those from SR-RAC, GT-CON and IM-CON, possibly due to differences in moisture and lipid contents (Table 5). The combination of the two principal components categorized the treatments into three distinct groups, i.e. SR-CON, GT-RAC and IM-RAC; GT-CON and IM-CON; and SR-RAC. This arrangement separated each RAC treatment from their respective CON counterpart. GT-CON and IM-CON were more distant from their RAC counterparts than SR-CON and SR-RAC (Fig. 1), indicating that dietary ractopamine influenced the quality parameters of frankfurters from GT and IM at a greater degree than those from SR.

3.1.5 Conclusions

The present study indicated that the effects of dietary ractopamine on the quality attributes of pork frankfurters were different in gilts, immunocastrated male pigs, and surgically castrated male pigs. Ractopamine demonstrated sex-specific effects on pork frankfurters and, to a limited extent, nullified the negative effects of immunocastration on

the quality of pork frankfurters. Since more than three-fourths of pork is retailed and consumed as processed meats, swine industry may adopt sex-specific dietary strategies to improve pork quality.

3.1.6 Acknowledgment

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Table 1: Formulation of pork frankfurters

Ingredient	% w/w
Meat	52.7
Pork fat	25.0
Ice	18.0
Maltodextrin	1.8
Sodium chloride	1.6
Sodium erythorbate	0.3
Sodium nitrite	0.2
Seasonings	0.4
Total	100%

Table 2: Effect of dietary ractopamine and sex on physico-chemical parameters of meat from deboned hams

	Treatments*						
	CON			RAC			
Parameters	GT	SR	IM	GT	SR	IM	SEM
pH	5.90 [b]	6.00 [a]	5.72 [c]	6.03 [a]	6.02 [a]	5.88 [b]	0.02
a_w	0.9927 [b]	0.9953 [a]	0.9933 [ab]	0.9947 [ab]	0.9933 [ab]	0.9943 [ab]	0.0007
Moisture (%)	69.03 [ab]	70.09 [ab]	68.26 [b]	72.61 [a]	71.15 [a]	70.99 [a]	0.78
Protein (%)	19.33 [b]	18.25 [d]	18.84 [c]	19.43 [b]	19.85 [a]	19.63 [ab]	0.15
Lipid (%)	9.51 [b]	9.87 [b]	11.67 [a]	6.81 [d]	9.99 [b]	8.29 [c]	0.13

SEM = Standard error of the mean

[a–d] Means in a row without common superscripts are different ($P < 0.05$).

* CON = 0 ppm ractopamine diet; RAC = 7.5 ppm ractopamine diet; GT = gilts; SR = surgically castrated male pigs; IM = immunocastrated male pigs

Table 3: Effect of sex on physico-chemical parameters of pork frankfurters

	Treatments*			
Parameters	GT	SR	IM	SEM
Cooking yield (%)	91.85 [b]	91.90 [b]	93.45 [a]	0.34
pH	6.21 [ab]	6.23 [a]	6.18 [b]	0.01
Protein (%)	12.7	12.54	12.58	0.10

SEM = Standard error of the mean
[a–b] Means in a row without common superscripts are different ($P < 0.05$).
* GT = gilts; SR = surgically castrated male pigs; IM = immunocastrated male pigs

Table 4: Effects of dietary ractopamine on physico-chemical parameters of pork frankfurters

	Treatments*		
Parameters	CON	RAC	SEM
Cooking yield (%)	92.10	92.70	0.28
pH	6.17 [b]	6.24 [a]	0.01
Protein (%)	12.41[b]	12.80 [a]	0.08

SEM = Standard error of the mean
[a–b] Means in a row without common superscripts are different ($P < 0.05$).
* CON = 0 ppm ractopamine diet; RAC = 7.5 ppm ractopamine diet

Table 5: Effect of dietary ractopamine and sex on proximate composition of pork frankfurters

	Treatments*						
	CON			RAC			
Parameters	GT	SR	IM	GT	SR	IM	SEM
a_w	0.9773 ^{ab}	0.9737 ^{c}	0.9760 ^{abc}	0.9797 ^{a}	0.9760 ^{abc}	0.9743 ^{bc}	0.0007
Moisture (%)	53.28 ^{d}	53.94 ^{c}	54.56 ^{b}	56.68 ^{a}	54.26 ^{bc}	56.15 ^{a}	0.11
Lipid (%)	29.50 ^{a}	28.44 ^{a}	28.68 ^{a}	25.55 ^{b}	28.28 ^{a}	25.96 ^{b}	0.21

SEM = Standard error of the mean
$^{a–d}$ Means in a row without common superscripts are different ($P < 0.05$).
* CON = 0 ppm ractopamine diet; RAC = 7.5 ppm ractopamine diet; GT = gilts; SR = surgically castrated male pigs; IM = immunocastrated male pigs

Table 6: Effect of dietary ractopamine and sex on instrumental color of pork frankfurters

	Treatments*						
	CON			RAC			
Parameters	GT	SR	IM	GT	SR	IM	SEM
*L**	75.23 ^{a}	73.69 ^{c}	74.92 ^{a}	73.87 ^{bc}	74.69 ^{a}	74.33 ^{b}	0.13
*a**	4.41 ^{bc}	4.66 ^{ab}	5.05 ^{a}	5.22 ^{a}	4.12 ^{c}	4.56 ^{abc}	0.13
*b**	11.37 ^{abcd}	11.93 ^{ab}	10.64 ^{d}	11.39 ^{c}	11.2 ^{bcd}	12.19 ^{a}	0.18
Chroma	12.23 ^{bc}	12.94 ^{a}	11.79 ^{c}	12.56 ^{b}	12.3 ^{bc}	12.7 ^{ab}	0.13
Hue	65.69 ^{bc}	68.75 ^{b}	64.28 ^{c}	65.18 ^{c}	70.92 ^{a}	70.42 ^{a}	0.73

SEM = Standard error of the mean

$^{a–d}$ Means in a row without common superscripts are different ($P < 0.05$).

* CON = 0 ppm ractopamine diet; RAC = 7.5 ppm ractopamine diet; GT = gilts; SR = surgically castrated male pigs; IM = immunocastrated male pigs

Table 7: Effect of ractopamine and sex on instrumental texture parameters of pork frankfurters

	Treatments*						
	CON			RAC			
Parameters	GT	SR	IM	GT	SR	IM	SEM
Hardness (N)	20.50 ^{bc}	22.49 ^{ab}	17.66 ^{c}	18.25 ^{c}	21.39 ^{abc}	25.11 ^{a}	0.90
Springiness	0.81 ^{c}	0.83 ^{abc}	0.81 ^{c}	0.85 ^{ab}	0.82 ^{bc}	0.85 ^{a}	0.01
Cohesiveness	0.62 ^{a}	0.58 ^{ab}	0.52 ^{b}	0.52 ^{b}	0.58 ^{ab}	0.60 ^{ab}	0.02
Resistance	0.32 ^{a}	0.30 ^{ab}	0.25 ^{b}	0.27 ^{ab}	0.29 ^{ab}	0.32 ^{a}	0.01
Shear force (N)	6.57 ^{c}	10.00 ^{a}	6.08 ^{c}	9.02 ^{ab}	8.34 ^{b}	6.87 ^{c}	0.26

SEM = Standard error of the mean

$^{a–c}$ Means in a row without common superscripts are different ($P < 0.05$).

* CON = 0 ppm ractopamine diet; RAC = 7.5 ppm ractopamine diet; GT = gilts; SR = surgically castrated male pigs; IM = immunocastrated male pigs

Table 8: Effect of dietary ractopamine on sensory attributes of pork frankfurters

	Treatments*		
Attributes	CON	RAC	SEM
Appearance	6.52	6.69	0.13
Odor	6.79	6.99	0.12
Texture	6.69 [b]	7.07 [a]	0.13
Flavor	6.85	7.08	0.13
Overall acceptance	6.87	7.02	0.11
Purchase intention	3.62	3.79	0.09

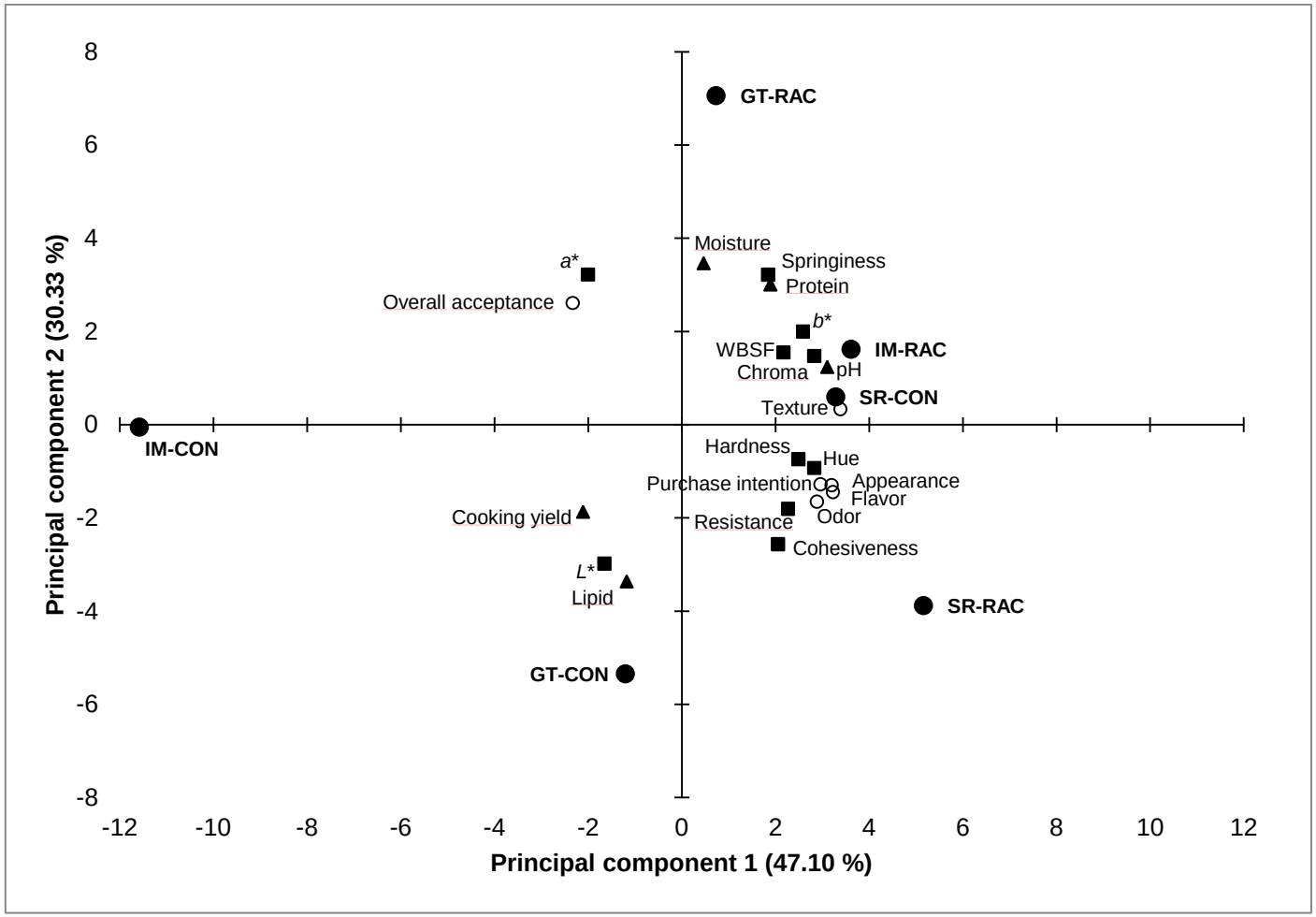
SEM = Standard error of the mean
[a–b] Means in a row without common superscripts are different ($P < 0.05$).
* CON = 0 ppm ractopamine diet; RAC = 7.5 ppm ractopamine diet

Table 9: Effect of sex on sensory attributes of pork frankfurters

	Treatments*			
Attributes	GT	SR	IM	SEM
Appearance	6.67	6.82	6.32	0.16
Odor	6.91	6.96	6.8	0.14
Texture	6.87	7.14	6.63	0.15
Flavor	6.89 [b]	7.38 [a]	6.63 [b]	0.16
Overall acceptance	6.97	6.76	7.11	0.14
Purchase intention	3.7 [ab]	3.97 [a]	3.44 [b]	0.11

SEM = Standard error of the mean
[a–b] Means in a row without common superscripts are different ($P < 0.05$).
* GT = gilts; SR = surgically castrated male pigs; IM = immunocastrated male pigs

Figure 1: Instrumental, sensory, and physico-chemical data of pork frankfurters in the plane defined by two principal components



3.2 CHAPTER 2: DIETARY RACTOPAMINE INFLUENCES SARCOPLASMIC PROTEOME PROFILE OF PORK LONGISSIMUS THORACIS [Ψ]. Under review - *Meat Science*, (Paper 2)

Bruno R. C. Costa-Lima [a, b], Surendranath P. Suman [a, *], Shuting Li [a], Carol M. Beach [c], Teofilo J. P. Silva [b], Expedito T. F. Silveira [d], Benjamin M. Bohrer [e, 1], Dustin D. Boler [e, 1]

[a] Department of Animal and Food Sciences, University of Kentucky, Lexington, KY 40546, USA

[b] Departamento de Tecnologia de Alimentos, Faculdade de Veterinaria, Universidade Federal Fluminense, Niteroi, 24230-340, Rio de Janeiro, Brazil

[c] Proteomics Core Facility, University of Kentucky, Lexington, KY 40506, USA

[d] Centro de Tecnologia de Carnes, Instituto de Tecnologia de Alimentos, Campinas, 13070-178, Sao Paulo, Brazil

[e] Department of Animal Sciences, The Ohio State University, Columbus, OH 43210, USA

[Ψ] Approved for publication as journal article number 14-07-007 by Director of Kentucky Agricultural Experiment Station.

* Corresponding author. Tel: +1-859-257-3248; E-mail: spsuma2@uky.edu (S. P. Suman).

[1] Current Address: Department of Animal Sciences, University of Illinois, Urbana, IL 61801, USA

3.2.1 Abstract

Dietary ractopamine improves pork leanness, whereas its effect on muscle proteome has not been characterized. We examined the influence of ractopamine on sarcoplasmic proteome of post-mortem pork *Longissimus thoracis* muscle. *Longissimus thoracis* samples were collected from carcasses (24 h post-mortem) of purebred Berkshire barrows (n = 9) managed in mixed-sex pens and fed finishing diets containing ractopamine (RAC; 7.4 ppm for 14 days followed by 10.0 ppm for 14 days) or without ractopamine for 28 days (CON). Sarcoplasmic proteome was analyzed using two-dimensional electrophoresis, tandem mass spectrometry, and data mining. Nine protein

spots were differentially abundant between RAC and CON groups. Glyceraldehyde-3-phosphate dehydrogenase and phosphoglucomutase-1 were over-abundant in CON, whereas serum albumin, carbonic anhydrase 3, L-lactate dehydrogenase A chain, fructose-bisphosphate aldolase A, and myosin light chain 1/3 were over-abundant in RAC. These results suggest that ractopamine influences the abundance of enzymes involved in glycolytic metabolism. The differential abundance of glycolytic enzymes could potentially influence the conversion of muscle to meat.

Keywords: Ractopamine; Sarcoplasmic proteome; *Longissimus thoracis*; Glycolysis

3.2.2 Introduction

Dietary strategies have been widely used to improve pork leanness (Dunshea, 2012). Ractopamine is a beta-agonist feed additive used in finishing diet to improve growth rate, feed efficiency, carcass yield, and leanness in pigs (Apple et al., 2007). The increase in leanness due to ractopamine is attributed to protein accretion (Bergen et al., 1989) and lipolysis (Mills, Spurlock, & Smith, 2003). Furthermore, ractopamine increases glucose turnover (Dunshea, Leury, Tilbrook, & King, 1998). The mode of action of ractopamine in skeletal muscle is through direct activation of beta-adrenergic receptors resulting in a shift from slow-twitch to fast-twitch muscle fibers and altering the proportion of muscle fiber composition to a fast-contracting glycolytic type (Aalhus, Schaefer, Murray, & Jones, 1992; Depreux, Grant, Anderson, & Gerrard, 2002; Gunawan, Richert, Schinckel, Grant, & Gerrard, 2007).

Fresh meat quality is influenced by muscle source as well as fiber type (Chang et al., 2003; Choe et al., 2008; Lee et al., 2012). Bowker, Grant, Forrest, and Gerrard (2000) reported that the muscles composed mainly of white fibers (type IIB) exhibit greater myofibrillar ATP-ase activity and predominantly anaerobic metabolism than the muscles composed primarily of red fibers (type I and IIA). In pigs, the *Longissimus* muscle is mainly (more than 80%) composed of type IIB white fibers, and therefore have predominantly glycolytic metabolism (Larzul et al., 1997). Previous investigations reported that the predominance of type IIB white fibers influences pork quality attributes

such as tenderness and water-holding capacity (Kim et al., 2008; Ryu, Lee, Lee, & Kim, 2006).

Several investigations examined the influence of dietary ractopamine on fresh pork quality (Apple et al., 2007; Boler et al., 2011; Kutzler et al., 2011; Lanferdini, Lovatto, Melchior, Orlando, Ceccantini, & Poleze, 2013). The effects of ractopamine on pork quality have been primarily attributed to an increase in myofibrillar protein synthesis and improved carcass yield and cutability (Adeola, Ball, & Young, 1992; Bohrer et al., 2012; Carr et al., 2009; Kutzler et al., 2011). The sarcoplasmic proteome comprises soluble proteins and enzymes, constitutes approximately one-third of the total proteins in skeletal muscles, and governs the biochemical processes influencing muscle metabolism (Scopes, 1970). Furthermore, the conversion of muscle to meat involves drastic shifts in metabolism, in which sarcoplasmic proteome plays a critical role (Jia et al., 2006). However, studies were not undertaken on the effect of ractopamine on sarcoplasmic proteome in pork muscles. Therefore, the objective of this study was to examine the influence of dietary ractopamine on the sarcoplasmic proteome profile of *Longissimus thoracis* muscle in pigs.

3.2.3 Materials and methods

3.2.3.1 Animal production and carcass fabrication

The animal care protocol for the experiment was reviewed and approved by Institutional Animal Care and Use Committee at The Ohio State University (Columbus, OH, USA). Two-hundred purebred Berkshire pigs (barrows and gilts) with an average initial body weight of 68.9 kg were used as previously described (Bohrer, Kyle, Little, Zerby, & Boler, 2013), and all the animals were raised under similar conditions at The Ohio State University Western Agricultural Research Station. The pigs were stratified over two blocks and housed in mixed-sex pens, and pens served as replicates in this experiment. Each block consisted of ten pens (five pens x 2 dietary treatments). Within each dietary treatment four pens had six barrows and four gilts, whereas one pen

contained five barrows and five gilts. Overall pen size was 16.25 m^2 (including 3.9 m^2 of slatted floor area), and thus each pig received approximately 1.63 m^2 of floor space. Each pen had a double nipple water drinker and a 4-hole single-sided box feeder that provided a total of 122 cm of linear feeder space (12.2 cm/pig). Pigs were housed in a curtain-sided, naturally ventilated barn and were provided ad libitum access to feed and water throughout the finishing trial. Animals were allotted by bodyweight and provided a 14-day allocation period prior to the start of the treatment diets. Within each block, animals in five pens were finished on a step-up ractopamine diet (RAC; 17.1% crude protein, 1.04% total lysine) with 7.4 mg/kg ractopamine for 14 d followed by 10 mg/kg ractopamine for the last 14 d prior to harvest, whereas the pigs in the other five pens were finished on a control diet (CON; 13.1% crude protein, 0.76% total lysine) with 0 mg/kg ractopamine. Thus RAC and CON treatments each had one-hundred pigs in ten pens from two blocks. Diets were analyzed to ensure ractopamine inclusion levels were within acceptable tolerances (75–125%) of the claim for each diet.

Costa-Lima et al. (2014) recently reported that the effect of ractopamine on color and textural attributes of pork frankfurters is sex-specific. Therefore, to avoid any potential effect of sex on muscle proteome, only barrows were selected for harvesting samples for proteome analysis. At the end of the 28-day feeding period, one barrow (105 kg average live body weight) was randomly selected from nine pens in CON and RAC treatments, and these eighteen animals were transported to The Ohio State University Meat Science Laboratory. This approach provided nine replicates (n = 9) for proteome analysis. The pigs were kept overnight in lairage with free access to water, but with no access to feed. The animals were humanely harvested using electrical stunning and exsanguination, and the carcasses were chilled for 24 h at 4°C. From the right side of the carcasses, a 2.54-cm loin chop (*Longissimus thoracis* muscle) was cut at the 10^{th} rib. The muscle samples were individually vacuum-packaged and frozen at –80°C. Frozen muscle samples were transported in dry ice to the University of Kentucky (Lexington, KY, USA), where they were stored at –80°C until proteome analysis.

3.2.3.2 Isolation of sarcoplasmic proteome

Sarcoplasmic proteome was extracted according to Joseph, Suman, Rentfrow, Li, and Beach (2012). Five grams of frozen muscle samples were homogenized in 25 ml ice-cold extraction buffer (40 mM Tris, 2mM EDTA, and pH 8.0). The homogenate was centrifuged at 10,000 x *g* for 15 min at 4°C. The supernatant (sarcoplasmic proteome) was filtered and utilized.

3.2.3.3 Two-dimensional electrophoresis (2DE)

The protein concentration of the sarcoplasmic proteome extract was determined using Bradford assay (Bio-Rad, Hercules, CA, USA). An aliquot corresponding to 900 µg of protein was mixed with rehydration buffer optimized to 7 M urea, 2 M thiourea, 4% CHAPS, 20 mM DTT, 0.5% Bio-Lyte 5/8 ampholyte (Bio-Rad), and 0.001% Bromophenol blue, and was loaded onto immobilized pH gradient (IPG) strips (pH 5–8, 17 cm). Gels were subjected to passive rehydration for 16 h, and then subjected to first-dimension isoelectric focusing (IEF) in a Protean IEF cell system (Bio-Rad) applying a linear increase in voltage initially and a final rapid voltage ramping to reach a total of 80 kVh. Subsequently the IPG strips were equilibrated in SDS-containing buffers, first with equilibration buffer I (containing 6 M urea, 0.375 M Tris-HCl, pH 8.8, 2% SDS, 20% glycerol, 2% DTT) followed by equilibration buffer II (containing 6 M urea, 0.375 M Tris-HCl, pH 8.8, 2% SDS, 20% glycerol, 2% DTT, 2.5% iodoacetamide), each for 15 min. The proteins were resolved in the second dimension on 12% SDS–PAGE (38.5:1 ratio of acrylamide to bis-acrylamide) using Protean II XL system (Bio-Rad). The gels were stained using Colloidal Coomassie Blue for 48 h and destained until background was cleared. Samples of both treatments (CON and RAC) were run under the same conditions. Two gels per animal were produced resulting in a total of 36 gels.

3.2.3.4 Gel image analysis

Digital images of the stained gels were captured using Versa Doc (Bio-Rad) and analyzed using PDQuest (Bio-Rad). Images were first subjected to automatic spot

detection and matching optimized by the aid of landmark protein spots, and the matched spots were normalized (Meunier et al., 2005). A protein spot was considered to be differentially abundant when it was associated with 1.5-fold intensity difference and with 5% significance level ($P < 0.05$) in a pairwise Student's t test as described by Joseph et al. (2012).

3.2.3.5 Protein identification by mass spectrometry

The spots exhibiting differential abundance between the treatments were excised from the gel using pipet tips, placed in microtubes for destaining by two 30-min washes with 50 mM NH_4HCO_3/ 50% CH_3CN, vortexed for 10 min, and dried in a vacuum centrifuge. The respective spot was excised from the counterpart treatment to confirm the match. Proteins in the gel fragment were reduced by reaction with 10 mM DTT in 50 mM NH_4HCO_3 solution and incubation at 57°C for 30 min. The supernatant was discarded, and the proteins (present at the gel piece) were alkylated by addition of 50 mM NH_4HCO_3 containing 50 mM iodoacetamide and incubated for 30 min at 25°C without exposure to light. Further, the gel piece was washed twice with 50 mM NH_4HCO_3 and once with CH_3CN, and then partially dried in a vacuum centrifuge. The dried gel piece was rehydrated with a solution of 40 mM NH_4HCO_3 / 9% CH_3CN, containing 20 ng/µL of proteomic grade trypsin (Sigma, St. Louis, MO, USA) on ice for 1 h. An additional volume of 40 mM NH_4HCO_3/9% CH_3CN was added to cover the sample, and the microtube was incubated for 18 h at 37°C. Peptides were extracted from the gel piece in 0.1 % trifluoroacetic acid by sonication for 10 min followed by vortexing for 10 min, and then the extraction was repeated using a solution on 50% acetonitrile containing 0.1% trifluoroacetic acid. The extracts were combined, and the volume was reduced to remove most of the acetonitrile. Peptide extracts were desalted and concentrated using solid phase extraction using 1 mm of Empore C-18 (3M, St. Paul, MN, USA) packed in a 0.1–10 µL pipet tip (Sarstedt, Newton, NC, USA). The peptides were eluted in 5 µL of 50 % CH_3CN/0.1% trifluoroacetic acid.

An aliquot of 0.3 µL of the desalted peptide extract was spotted onto an Opti-ToF 384 well insert (Applied Biosystems, Foster City, CA, USA) with 0.3 µL of 5 mg/mL α-

cyano-4-hydroxycinnamic acid (Aldrich, St. Louis, MO, USA) in 50% CH_3CN/0.1% trifluoroacetic acid. Crystallized samples were washed with cold 0.1% trifluoroacetic acid and were analyzed using a 4800 MALDI TOF-TOF Proteomics Analyzer (Applied Biosystems, Foster City, CA, USA). An initial MALDI MS spectrum was acquired for each spot (400 laser shots per spectrum), and a maximum of 15 peaks with a signal-to-noise ratio of more than 20 were automatically selected for MS-MS analysis (1000 laser shots per spectrum) by post-source decay. Peak lists from the MS-MS spectra were submitted for database similarity search using Protein Pilot 2.0 (Applied Biosystems, Foster City, CA, USA), and the search was performed in the UniProt database to identify the proteins.

3.2.4 Results and discussion

The image analyses of the sarcoplasmic proteome gels identified nine differentially abundant spots (Figure 1). The identified proteins are listed in table 1 along with their accession number, score of the database search (ProtScore), numbers of matched peptides, and sequence coverage. The functional roles of these proteins are presented in table 2; the identified proteins were involved in glycolysis, chaperone, transport, and muscle contraction. Two protein spots (glyceraldehyde-3-phosphate dehydrogenase and phosphoglucomutase-1) were over-abundant in CON, whereas five spots (serum albumin, carbonic anhydrase 3, L-lactate dehydrogenase A chain, fructose-bisphosphate aldolase A, and myosin light chain 1/3) were over-abundant in RAC. In addition, two distinct protein spots were identified as stress-induced-phosphoprotein 1; while one spot was over-abundant in CON, the other demonstrated over-abundance in RAC.

3.2.4.1 Proteins over-abundant in CON

3.2.4.1.1 *Glyceraldehyde-3-phosphate dehydrogenase*

Glyceraldehyde-3-phosphate dehydrogenase was over-abundant in CON samples (Tables 1 and 2). This glycolytic enzyme reversibly converts glyceraldehyde-3-

phosphate and NAD^+ to 1,3-bisphosphoglycerate and NADH. Mammalian glyceraldehyde-3-phosphate dehydrogenase is considered a multifunctional protein involved in several cytoplasmic and nuclear pathways (Sirover, 2011; Sirover, 1999). The over-abundance of glyceraldehyde-3-phosphate dehydrogenase in CON was unexpected and not readily explained since dietary ractopamine is known to induce a muscle fiber type shift towards a more glycolytic phenotype (Depreux et al., 2002). Further investigations are necessary to explain this observation on the basis of possible translocation of the enzyme between sarcoplasm and nuclei (Sirover, 2005) in skeletal muscles.

Previous research reported the relationship between glyceraldehyde-3-phosphate dehydrogenase and pork quality traits. Kwasiborski et al. (2008) correlated sarcoplasmic proteome profile of longissimus muscles to pork quality and documented that glyceraldehyde-3-phosphate dehydrogenase was positively correlated to the carcass temperature at 45 min post-mortem, ultimate pH at 24 h, and drip loss at 72 h post-mortem. Moreover, Park, Kim, Lee, and Hwang (2007) investigated the longissimus fiber type and reported that glyceraldehyde-3-phosphate dehydrogenase is a potential indicator of post-mortem proteolysis in pork.

3.2.4.1.2 *Phosphoglucomutase-1*

Phosphoglumutase-1 was over-abundant in CON samples (Tables 1 and 2). This glycolytic enzyme catalyzes the transfer of phosphate between glucose-1-phosphate and glucose-6-phosphate (Cori, Colowick, & Cori, 1938). The over-abundance of phosphoglucomutase-1 in CON loins was unexpected, similar to the observation in glyceraldehyde-3-phosphate dehydrogenase. Further studies are necessary to characterize the mechanisms through which ractopamine decreases the abundance of phosphoglucomutase-1 and at the same time stimulates a shift to glycolytic muscle phenotype (Depreux et al., 2002). Previous studies reported the existence of correlation between phosphoglucomutase-1 and pork quality traits. Zelechowska, Przybylski, Jaworska, and Sante-Lhoutellier (2012) investigated the protein profile of the purge from pork loins and observed that phosphoglucomutase-1 was over-abundant in pale, soft,

and exudative samples. Furthermore, these authors documented that phosphoglucomutase-1 was negatively correlated to meat pH and positively correlated with *L** value and drip loss. Kwasiborski et al. (2008) examined the influence of the sarcoplasmic proteins on pork loin quality and reported that phosphoglucomutase-1 was negatively correlated to *a** value and positively correlated to thawing loss.

3.2.4.1.3 *Stress-induced-phosphoprotein 1*

Two spots (spots 3 and 4 in Figure 1) were identified as stress-induced-phosphoprotein 1 (Table 1). While spot 3 was over-abundant in CON, spot 4 was over-abundant in RAC samples (Table 2). Stress-induced-phosphoprotein 1 is also known as heat shock protein (HSP)-organizing protein. As a co-chaperone, stress-induced-phosphoprotein 1 links the chaperones HSP70 and HSP90 together and modulates their activities (Odunuga, Longshaw, & Blatch, 2004). The two deferentially abundant protein spots identified as stress-induced-phosphoprotein 1 exhibited similar molecular weight but different isoelectric points (Figure 1). This shift in the isoelectric point could be possibly due to phosphorylation of stress-induced-phosphoprotein 1, which has been previously reported (Masaoka, Nishi, Ryo, Endo, & Sawasaki, 2008). Furthermore, these authors concluded that phosphorylation influences the cellular localization of stress-induced-phosphoprotein 1. Phosphorylation of proteins leads to shift in the isoelectric point (Peck, 2006) as we observed in figure 1.

Stress-induced-phosphoprotein 1 has previously been identified as possible biomarker for meat quality. Di Luca, Elia, Hamill, and Mullen (2013) investigated the proteome profile of longissimus exudate and observed that pork loins exhibiting increased drip loss (comparable to pale, soft, and exudative) as well as those exhibiting decreased drip loss (similar to dark, firm, and dry) contained lower abundance of stress-induced-phosphoprotein 1. On the other hand, stress-induced-phosphoprotein 1 was positively correlated to color stability in beef longissimus steaks (Joseph et al. 2012).

3.2.4.2 Proteins over-abundant in RAC

3.2.4.2.1 *Serum albumin*

Serum albumin (Table 1) was over-abundant (Table 2) in RAC. This highly soluble plasma protein has several important biological functions; it is a binding protein as well as antioxidant (Roche, Rondeau, Singh, Tarnus, & Bourdon, 2008). The porcine serum albumin shares 76% homology with the human serum albumin, which is a predominant antioxidant in plasma and is responsible for scavenging more than 70% of the free radicals through its multiple-binding sites. In addition, the ability of serum albumin to bind copper and iron is critical to the protein's antioxidant functions (Halliwell, 1988). Furthermore, serum albumin can bind with polyunsaturated fatty acids and lipid-soluble antioxidants, facilitating their interactions, thereby protecting the fatty acids against oxidative damage (Roche et al., 2008; More, & Bulmer, 2013). The presence of serum albumin in sarcoplasmic proteome was reasonable because muscle tissue was homogenized in buffer for the extraction of proteins; water-soluble plasma proteins from the residual blood trapped within the muscle are also extracted in this process. The over-abundance of serum albumin in RAC could be a physiological adaptation to the increase in protein accretion and lipolysis in ractopamine-fed animals (Bergen et al., 1989; Mills et al., 2003). The increased serum albumin level could be a mechanism to protect the proteins from the reactive products of lipolysis because protein and lipid stabilities in muscle foods are closely interrelated (Decker, Livisay, & Zhou, 2000).

Several previous proteomic investigations documented the presence of serum albumin in pork muscles. On their attempts to identify biomarkers for pork quality, Hwang et al. (2005) and Sayd et al. (2012) reported the presence of serum albumin in the proteome of post-mortem longissimus muscles. Moreover, Kwasiborski et al. (2008) reported that serum albumin was negatively correlated with cooking loss and pH and temperature at 45 min in pork longissimus muscles. In addition, Mule et al. (2006) evaluated the dietary amino acid restrictions in pigs and reported that the high protein diet promoted an increase in the serum albumin content. Based on these reports, the observed over-abundance of serum albumin in RAC group may also be partially

attributed to the greater protein and lysine contents in the finishing diet compared to CON.

3.2.4.2.2 *Carbonic anhydrase-3*

Carbonic anhydrase-3, also known as carbonate dehydratase, was over-abundant on RAC samples (Table 2). A member of carbonic anhydrase family, this protein differs from the other isozymes with respect to its low hydratase activity and resistance against sulfonamide, a carbonic anhydrase inhibitor (Gros & Dodgson, 1988). In addition, a recent report demonstrated that carbonic anhydrase-3 decreases adipogenic activity through down-regulation of peroxisome proliferator-activated receptor-γ2, an adipogenic gene (Mitterberger, Kim, Rostek, Levine, & Zwerschke, 2012). Therefore, the repartitioning effect of dietary ractopamine (Moody, Hancock, & Anderson, 2000) appears to be correlated to the down-regulation of peroxisome proliferator-activated receptor-γ2 by the over-abundance of carbonic anhydrase-3.

Hwang et al. (2005) evaluated the influence of the postmortem proteome on quality attributes of pig longissimus muscle and concluded that carbonic anhydrase was a possible protein biomarker for predicting pork quality. In support, Kwasiborski et al. (2008) documented a negative correlation between carbonic anhydrase-3 and thawing loss in chops from barrows. Additionally, Damon et al. (2013) observed that gene expression of carbonic anhydrase-3 in pork longissimus muscle was negatively correlated with ultimate pH and positively with drip loss and *L** value.

3.2.4.2.3 *L-lactate dehydrogenase A chain*

L-lactate dehydrogenase A chain, an enzyme catalyzing the NADH-mediated reversible conversion of lactate to pyruvate (Gladden, 2004), was over-abundant in ractopamine-fed barrows (Table 2). Depreux et al. (2002) reported that ractopamine-fed pigs exhibited a skeletal muscle fiber type shift from oxidative (types IIA and IIX) to glycolytic (type IIB), which possibly increases the lactate dehydrogenase activity because glycolytic muscles (e.g. *Longissimus*) exhibit greater lactate dehydrogenase

activity than their oxidative (e.g. diaphragm) counterparts (Huber, Petzold, Rehfeldt, Ender, & Fiedler, 2007). However, Gunawan et al. (2007) did not observe any difference in lactate dehydrogenase gene expression in loins from ractopamine-fed pigs in comparison with controls. The difference between our results and those of Gunawan et al. (2007) could be due to the fact that while the genome is static, the proteome is dynamic (Peng, & Gygi, 2001) and constantly changes in skeletal muscles ante-mortem, peri-mortem, and post-mortem (Hollung, Veiseth, Jia, Faergestad, & Hildrum, 2007).

Ramanathan, Mancini, and Konda (2009) investigated the influence of lactate and lactate dehydrogenase on the oxygen consumption of beef mitochondria and reported that the production of NADH by lactate dehydrogenase increased the mitochondrial oxygen consumption resulting in dark colored meat. Therefore, the over-abundance of L-lactate dehydrogenase A chain in ractopamine-fed animals can possibly influence pork color (Ramanathan, Mancini, Joseph, & Suman, 2013). In partial agreement, Kwasiborski et al. (2008) reported a negative correlation between lactate dehydrogenase content and *L** value in pork loins. Furthermore, D'Alessandro et al. (2011) used proteomic tools to evaluate the pork quality of longissimus muscle and reported that lactate dehydrogenase content was correlated to pH at 24 h post-mortem.

3.2.4.2.4 Fructose-bisphosphate aldolase A

Fructose-bisphosphate aldolase A, which was over-abundant on RAC (Table 2), catalyzes reversible aldol cleavage of fructose 1, 6-bisphosphate to glyceraldehyde-3-phosphate and dihydroxyacetone phosphate (Scheffler, & Gerrard, 2007). This enzyme is critical in glycolysis as well as gluconeogenesis (Horecker, MacGregor, Singh, Melloni, & Pontremoli, 1981) and is well conserved within mammalian species (www.expasy.org). The abundance of fructose-bisphosphate aldolase A in cytoplasm is influenced by metabolic substrates; high glucose levels in cytoplasm results in an increase in the abundance of this enzyme (Mamczur, Gamian, Kolodziej, Dziegiel, & Rakus, 2013). Previous investigations reported that dietary ractopamine increases glucose turnover (Dunshea et al., 1998) and favors a muscle fiber type shift from oxidative to glycolytic metabolism (Depreux et al., 2002). Based on these studies, the

observed over-abundance of fructose-bisphosphate aldolase A could be attributed to the shift in muscle energy metabolism. Zelechowska et al. (2012) investigated the relationship between proteins in purge and color quality of pork longissimus and reported that aldolase A was positively correlated to *b** values. D'Alessandro et al. (2011) and Hwang et al. (2005) also documented the presence of fructose-bisphosphate aldolase A in pork longissimus in their studies, whereas its relationship with meat quality was not examined.

3.2.4.2.5 Myosin light chain 1/3

Myosin light chain (MLC) 1/3 (Table 1) was over-abundant in RAC (Table 2). MLC 1 and 3 are known as essential light chain or alkali isoforms and are present in fast-twitch skeletal muscle fibers (Bortolotto, Cellini, Stephenson, & Stephenson, 2000). Both isoforms are encoded by a single gene resulting in identical amino acid sequence for the most part with the exception of the amino-terminal length; MLC 1 contains 49 amino acids in the amino terminus, while MLC 3 contains only 8 amino acids (Barton, & Buckingham, 1985; Schiaffino, & Reggiani, 1996). The appearance of this myofibrillar protein in the sarcoplasmic fraction could be explained by possible cleavage of myosin at the neck region close to the head (Lametsch, Roepstorff, & Bendixen, 2002) releasing the MLC 1/3 from the actomyosin into the sarcoplasm (Lametsch et al., 2006). The observed over-abundance of MLC in post-mortem muscles from ractopamine-fed pigs suggests an increase in the myofibrillar protein accretion (Adeola, Ball, & Young, 1992) and a shift in muscle fiber type towards a fast-twitch type; both of which are expected as a result of ractopamine feeding (Aalhus et al., 1992; Depreux et al., 2002). Choi, Ryu, and Kim (2007) investigated the influence of MLC isoforms in the glycolytic metabolism of pork longissimus muscle and meat quality. These authors concluded that although the MLC isoforms influenced the metabolism pathways in post-mortem skeletal muscles, their effects on meat quality traits were limited. Similarly, Lametsch et al. (2003) reported no correlation between MLC 1 and Warner−Bratzler shear force of pork longissimus muscle. On the other hand, Kwasiborski et al. (2008) evaluated the influence of sarcoplasmic proteome on the meat quality traits of pork longissimus muscle and

reported a negative correlation between MLC 1 and drip loss at 72 h post-mortem. Moreover, Hwang, Park, Kim, Cho, & Lee (2005) documented that MLC 1 exhibited a positive correlation with Warner−Bratzler shear force value and a negative correlation with lightness in pork longissimus.

3.2.4.3 Conclusions

The results of the present study suggest that dietary ractopamine influenced the abundance of glycolytic enzymes and chaperones in sarcoplasmic proteome of post-mortem pork *Longissimus thoracis* muscles. Further studies are necessary to characterize how ractopamine feeding influences the sarcoplasmic proteome in ante- and peri-mortem muscles to elucidate the influence of this beta-agonist on muscle to meat conversion in pigs.

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Table 1 – Differentially abundant sarcoplasmic proteins in *Longissimus thoracis* muscle of pigs fed on ractopamine.

Spot [a]	Accession number	Protein	ProtScore/ matched peptides	Sequence coverage (%)
1	P00355	Glyceraldehyde-3-phosphate dehydrogenase	10.95/8	29.7
2	Q08DP0	Phosphoglucomutase-1	22.00/12	29.5
3	Q3ZBZ8	Stress-induced-phosphoprotein 1	18.98/9	23.9
4	Q4R8N7	Stress-induced-phosphoprotein 1	18.39/10	23.6
5	P08835	Serum albumin	20.31/10	23.2
6	Q5S1S4	Carbonic anhydrase 3	16.21/9	43.5
7	P00339	L-lactate dehydrogenase A chain	14.14/8	24.4
8	Q5NVR5	Fructose-bisphosphate aldolase A	14.04/8	37.6
9	A0JNJ5	Myosin light chain 1/3	16.03/11	52.1

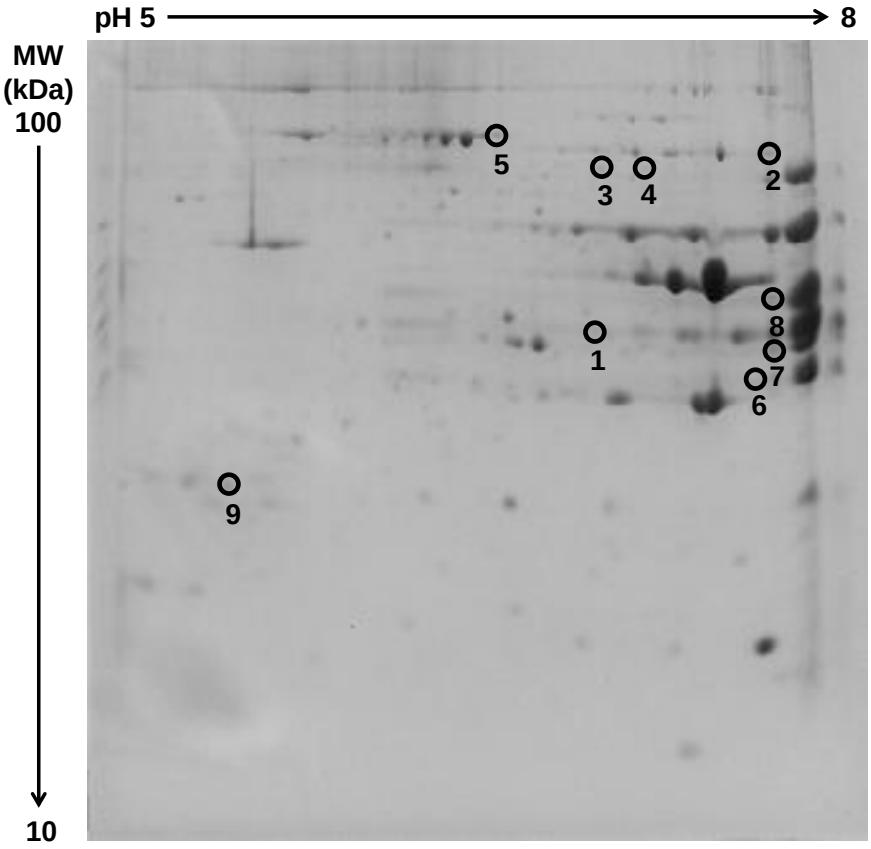
[a] Spot number refers to the numbered spots in gel image (Figure 1)
For each spot, parameters related to protein identification are provided – UniProt accession number; ProtScore and number of matched peptides; sequence coverage of peptides in tandem mass spectrometry

Table 2 – Functional roles of differentially abundant sarcoplasmic proteins in *Longissimus thoracis* muscle of pigs fed on ractopamine.

Spot [a]	Protein	Function	Over-abundant treatment [b]	Spot ratio
1	Glyceraldehyde-3-phosphate dehydrogenase	Glycolysis	CON	1.56 [c]
2	Phosphoglucomutase-1	Glycolysis	CON	1.85 [c]
3	Stress-induced-phosphoprotein 1	Chaperone	CON	1.64 [c]
4	Stress-induced-phosphoprotein 1	Chaperone	RAC	1.64 [d]
5	Serum albumin	Transport	RAC	2.00 [d]
6	Carbonic anhydrase 3	Hydration of CO_2	RAC	2.63 [d]
7	L-lactate dehydrogenase A chain	Glycolysis	RAC	1.75 [d]
8	Fructose-bisphosphate aldolase A	Glycolysis	RAC	1.52 [d]
9	Myosin light chain 1/3	Muscle contraction	RAC	1.56 [d]

[a] Spot number refers to the numbered spots in gel image (Figure 1)
[b] CON = 0 ppm ractopamine in finishing diet for 28 days; RAC = 7.4 ppm ractopamine in finishing diet for 14 days followed by 10.0 ppm for 14 days
[c] Spot ratio of CON/RAC
[d] Spot ratio of RAC/CON

Figure 1 – Coomassie-stained two-dimensional gel of the sarcoplasmic proteome extracted from pork *Longissimus thoracis* muscle. Nine proteins spots differentially abundant in control and ractopamine-fed pigs are encircled and numbered.



4 GENERAL CONCLUSIONS

The overall conclusion from this project is that dietary ractopamine stimulates lean meat accretion in skeletal muscle through inhibiting lipogenesis. This observation was supported by increased protein and decreased lipid contents in ham from ractopamine-fed pigs. Furthermore, ractopamine proved to be a suitable feed additive capable of suppressing the negative effects of immunocastration. Although the investigated sarcoplasmic proteome was from *Longissimus thoracis*, it is potentially safe to extrapolate that the observed differences in the protein abundances promoted by dietary ractopamine, did not negatively affect the protein quality of ham; both ham and loin are mainly composed by glycolytic fiber types. This can be corroborated as no negative effects were observed on frankfurters elaborated from raw materials of pigs fed on ractopamine.

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6 APPENDIX

6.1 PAPER 1

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Sex-specific effect of ractopamine on quality attributes of pork frankfurters

Bruno R.C. Costa-Lima [a,*], Anna C.V.C.S. Canto [a], Surendranath P. Suman [b], Carlos A. Conte-Junior [a], Expedito T.F. Silveira [c], Teofilo J.P. Silva [a]

[a] Departamento de Tecnologia de Alimentos, Faculdade de Veterinaria, Universidade Federal Fluminense (UFF), Niteroi 24230-340, Rio de Janeiro, Brazil
[b] Department of Animal and Food Sciences, University of Kentucky, Lexington, KY 40546, USA
[c] Centro de Tecnologia de Carnes, Instituto de Tecnologia de Alimentos, Campinas 13070-178, Sao Paulo, Brazil

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Sensory quality

ABSTRACT

Our objective was to determine the effect of dietary ractopamine and immunocastration on the quality attributes of pork frankfurters. Gilts (GT), surgically castrated male pigs (SR) and immunologically castrated male pigs (IM) were fed diets containing 7.5 ppm ractopamine (RAC) or no ractopamine (CON) for 21 days prior to harvest. Deboned hams were manufactured into frankfurters, and physico-chemical parameters, instrumental color and texture, and sensory attributes were evaluated. Ractopamine increased ($P < 0.05$) L^* (lightness) in SR, whereas it decreased ($P < 0.05$) lightness in IM and GT. While ractopamine increased ($P < 0.05$) a^* (redness) in GT, a reverse ($P < 0.05$) trend was observed in SR. With respect to instrumental texture, ractopamine increased ($P < 0.05$) hardness, resistance, and springiness in IM, cohesiveness and springiness in GT, and shear force in SR. These results indicated that ractopamine exerted sex-specific effects on frankfurter quality. Swine industry may adopt sex-specific dietary strategies to optimize the quality of further processed meat products.

1. Introduction

Boar taint is a major problem in pork industry caused by androstenone produced by testes (Patterson, 1968) and skatole generated by intestinal bacteria (Claus, Weiler, & Herzog, 1994). Surgical castration of piglets is employed to control boar taint (Prunier et al., 2006). However, this strategy decreases growth performance as it stops production of androgenic hormones. An alternative to surgical castration is immunocastration, which is achieved by immunizing male pigs against gonadotropin releasing factor (GnRF). Immunocastration decreases production of testicular hormones and minimizes accumulation of androstenone (Zamaratskaia et al., 2008). The decrease in testicular hormone enhances hepatic metabolism of skatole and decreases its accumulation (Doran, Whittington, Wood, & McGivan, 2002; Lin, Lou, & Squires, 2004a, 2004b). This approach allows entire male pigs to attain increased body weight (favored by testicular hormones), while preventing boar taint (Dunshea et al., 2001; Jaros et al., 2005). Immunocastration has been approved in swine production in more than 50 countries (Gispert et al., 2010). Previous investigations reported that immunocastrated male pigs exhibited meat quality attributes (instrumental and sensory) comparable to barrows and gilts (Boler et al., 2012; D'Souza & Mullan, 2002; Font i Furnols et al., 2008, 2009; Pauly, Luginbuhl, Ampuero, & Bee, 2012).

Ractopamine is a phenethanolamine repartitioning agent with beta-adrenergic agonist properties and is used to promote leanness in meat animals. The species-specific effects of ractopamine on protein metabolism and lipogenesis are well documented in meat-producing livestock (Avendano-Reyes et al., 2006; Dunshea, D'Souza, Pethick, Harper, & Warner, 2005; Liu & Mills, 1989; Lopez-Carlos et al., 2011). The effects of ractopamine on pork quality are associated with increased myofibrillar protein synthesis and improved carcass yield, cutability, and leanness (Adeola, Ball, & Young, 1992; Bohrer et al., 2012; Carr et al., 2009; Kutzler et al., 2011). Dietary ractopamine offers an excellent strategy to maximize the positive effects of immunocastration while controlling its negative effects such as increase in fat mass (Lanferdini et al., 2013). Therefore, a combination of immunocastration and ractopamine feeding has the potential to improve meat quality in pigs (Rikard-Bell et al., 2009).

In the modern-day meat industry, more than three-fourths of pork is further processed and marketed as ready-to-eat products such as ham, bacon, and sausage (Pork Checkoff, 2012). Thus it is important to determine the effect of ractopamine and immunocastration on further processed pork products. While several previous investigations focused on fresh pork quality, limited number of studies examined the effect of ractopamine and immunocastration on further processed pork products. Boler et al. (2011) and Font i Furnols et al. (2012) concluded that immunocastration does not necessarily influence quality parameters of hams. However, investigations are yet to be undertaken to evaluate the impact of ractopamine feeding and immunocastration on emulsion-type pork products. Frankfurters are pre-cooked emulsion-

* Corresponding author. Tel.: +55 21 2629 9545.
E-mail address: brunuff@gmail.com (B.R.C. Costa-Lima).

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type sausages having large market share. They are popular fast-food items and are widely consumed. Therefore, the aim of the present study was to evaluate the influence of dietary ractopamine on the sensory and instrumental attributes of pork frankfurters processed from immunocastrated male pigs, surgically castrated male pigs, and gilts.

2. Materials and methods

2.1. Animal production

The research protocols were in accordance with the guidelines of the Institutional Ethics Committee on Animal Use and the Ministry of Agriculture, Brazil. Ten female and twenty male piglets (AGPIC 337 male × CB22 female, Agroceres PIC, Sao Paulo, Brazil) were used in this study. All the animals were raised under similar conditions at a commercial swine production facility at Sao Paulo, Brazil. The piglets were selected when they were 5 days old and had a body weight of 1.5 kg. The sexes of the animals used in this study were gilts (GT), immunocastrated male pigs (IM), and surgically castrated male pigs (SR). Ten animals per sex were allocated. The animals were weaned at three weeks and were group-penned based on sex until 21 days prior to the harvest. Immunocastration was accomplished by vaccinating male pigs against GnRF with VIVAX (200 μg of GnRF–protein conjugate per ml of an aqueous adjuvant system; Pfizer, Brazil) at eight weeks and four weeks prior to slaughter. Surgical castration was performed when male piglets were seven days old.

The animals were finished for 175 days on a commercial diet to reach an average body weight of 115 kg. Twenty-one days before the harvest, five animals (n = 5) from each sex were randomly selected and fed a diet containing either 0 ppm (CON) or 7.5 ppm ractopamine (RAC; Paylean, Elanco Animal Health, Greenfield, IN, USA) until harvest. During those 21 days, the animals were group-penned based on six treatments (3 sexes × 2 diets). Thus the six treatments in this study were GT-CON, GT-RAC, IM-CON, IM-RAC, SR-CON, and SR-RAC. The animals were humanely harvested in a packing plant (Sao Paulo, Brazil) under Brazilian federal inspection. The carcasses were chilled at 2 °C for 24 h and were fabricated. The green hams (average weight of 9.8 kg) from the left side of the carcasses were deboned, and meat and fat were separated. The meat and fat from each deboned hams were separately bulk-packaged in polyvinyl chloride film, frozen at −18 °C, and transported to the pilot plant of the Instituto de Tecnologia de Alimentos (Campinas, Sao Paulo, Brazil) for further processing.

2.2. Frankfurter processing

Upon arrival at the Instituto de Tecnologia de Alimentos, each deboned ham was separately processed into frankfurters on the same day to provide five replicates (n = 5) per treatment. Frozen meat and fat were chopped to small pieces and were separately ground using a 5 mm plate. One 8-kg batch of emulsion was prepared from each deboned ham based on the formulation presented in Table 1. Meat was transferred to a bowl chopper, and salt was added. Meat was comminuted for 3 min at low speed to extract myofibrillar proteins until the temperature reached 6 °C, when other ingredients were slowly added. The temperature of the mixture was not allowed to exceed 12 °C. The emulsion was stuffed in cellulose casings (29 mm diameter) forming 100 g links. The frankfurter strings were placed in an industrial oven at 55 °C for 40 min for drying, and the temperature was increased 5 °C every 10 min until the oven temperature reached 80 °C. The frankfurters were cooked to an internal temperature of 75 °C. The internal temperature was monitored by thermocouples. The cooked frankfurters were cooled using a water shower (7 °C) for 10 min. The casings were peeled off manually using knife, and the frankfurters were vacuum packaged. The vacuum packaged frankfurters were heat-treated at 80 °C for 5 s, in a water bath, to decrease post-cooking bacterial contamination and were then stored at 4 °C until further analysis.

Table 1
Formulation of pork frankfurters.

Ingredient	% w/w
Meat	52.7
Pork fat	25.0
Ice	18.0
Maltodextrin	1.8
Sodium chloride	1.6
Sodium erythorbate	0.3
Sodium nitrite	0.2
Seasonings	0.4
Total	100%

2.3. Water activity, pH, and proximate composition

Frozen meat and cooked frankfurters were analyzed for pH, water activity (a_w), and proximate composition. The pH was measured using a probe pH meter (Model DM-21, Digimed, Sao Paulo, Brazil), whereas a_w was determined at 25 °C using Aqualab Decagon CX-2T water activity meter (Decagon, Pullman, WA, USA). The moisture, protein, and lipid contents were determined according to AOAC (2005).

2.4. Cooking yield

The initial weight of raw frankfurter strings was recorded. After cooking and cooling, the strings were blotted, and the final weight was measured. Cooking yield was calculated from differences in the initial and final weight and expressed as percentage of initial weight (Boles & Swan, 1996).

2.5. Instrumental color

Cooked frankfurters were bisected parallel to their long axis and maintained at 25 °C for 30 min before color evaluation. CIE L^* (lightness), a^* (redness), b^* (yellowness), hue, and chroma values were measured at two random locations on each sample using a Minolta CM-508d spectrophotometer (Osaka, Japan) with 8 mm diameter aperture, illuminant D65, and 10° standard observer (AMSA, 2012).

2.6. Instrumental texture

Shear force and texture profile (Bourne, 1978) of frankfurters were evaluated using TA-TX2i texture analyzer (Stable Micro System, Surrey, United Kingdom). Samples were cut into 2.5 cm length cylinders and held at 25 °C. For measuring shear force, Warner–Bratzler blade with a triangular notch was used. The samples were placed on the guillotine block and sheared completely as the blade moved down at the speed of 5 mm/s. For texture profile, a cylindrical metal probe of 35 mm diameter was used. The samples were compressed to 70% of their original height in two cycles at pre-test speed of 5 mm/s, test speed of 1 mm/s, and post-test speed of 5 mm/s. The time between the compressions was 2 s. The data on texture profile were obtained and processed by Texture Expert Software (Stable Micro System, Surrey, United Kingdom) and expressed as hardness, springiness, cohesiveness, and resistance.

2.7. Sensory evaluation

Consumer acceptance test was conducted to evaluate appearance, odor, texture, flavor, overall acceptance, and purchase intention. Forty-nine untrained panelists (21–30 years old) among students, faculty, and staff of the Instituto de Tecnologia de Alimentos were recruited. The panelists were regular consumers of frankfurters. The individual booths were equipped with computers. The coded samples were presented to panelists in randomized blocks and in a sequential monadic

Table 2
Effect of dietary ractopamine and sex on physico-chemical parameters of meat from deboned hams.

Parameters	Treatments[z]						SEM
	CON			RAC			
	GT	SR	IM	GT	SR	IM	
pH	5.90[b]	6.00[a]	5.72[c]	6.03[a]	6.02[a]	5.88[b]	0.02
a_w	0.9927[b]	0.9953[a]	0.9933[ab]	0.9947[ab]	0.9933[ab]	0.9943[ab]	0.0007
Moisture (%)	69.03[ab]	70.09[ab]	68.26[b]	72.61[a]	71.15[a]	70.99[a]	0.78
Protein (%)	19.33[b]	18.25[d]	18.84[c]	19.43[b]	19.85[a]	19.63[ab]	0.15
Lipid (%)	9.51[b]	9.87[b]	11.67[a]	6.81[d]	9.99[b]	8.29[c]	0.13

SEM = standard error of the mean.
[a–d]Means in a row without common superscripts are different ($P < 0.05$).
[z]CON = 0 ppm ractopamine diet; RAC = 7.5 ppm ractopamine diet; GT = gilts; SR = surgically castrated male pigs; IM = immunocastrated male pigs.

way. For each treatment, two 2-cm cuts of frankfurters were provided to the panelists. A nine-point hedonic scale (1 = dislike extremely; 2 = dislike very much; 3 = dislike moderately; 4 = dislike slightly; 5 = neither dislike nor like; 6 = like slightly; 7 = like moderately; 8 = like very much; 9 = like extremely) was used for appearance, odor, texture, flavor, and overall acceptance, whereas purchase intention was evaluated on a five-point scale (1 = certainly would not buy; 2 = probably would not buy; 3 = may or may not buy; 4 = probably would buy; 5 = certainly would buy). Drinking water and unsalted crackers were offered for cleaning the palate between samples (Stone & Sidel, 1993). Data were collected using the computers equipped with Compusense Five Version 4.2 software (Compusense Inc., Guelph, Ontario, Canada).

2.8. Statistical analysis

The experiment was a completely randomized design. The treatments were arranged as a full factorial of 3 sexes and 2 ractopamine levels. All the traits were analyzed using a linear model, which considered sex, ractopamine level, and their interactions as fixed effects. Each ham was considered as an experimental unit, and each one of the six treatments (3 sexes × 2 ractopamine levels) had five hams. Each deboned ham was individually processed into frankfurter to provide five replicates (n = 5) per treatment. Data for physico-chemical attributes, instrumental color, and instrumental texture were analyzed using the MIXED procedure (SAS, 2009). When the analysis of variance detected differences among the treatments at a significance level of 5%, the Tukey–Kramer test was applied to discriminate the least squares means. Sensory data were analyzed using ANOVA in Compusense Five Version 4.2 software, and the differences among means were detected at 5% level of significance using Tukey's HSD test.

Additionally, the data (physico-chemical attributes, instrumental color, instrumental texture, and sensory) were evaluated by principal component analysis in a correlation matrix using XLStat software (Addinsoft, Paris, France). In the correlation matrix, the data were centered and scaled based on the parameter means. The matrix consisted of 21 columns and 6 rows, with the columns representing the means of parameters and the rows representing treatments. For the sensory attributes, the scores from all the 49 panelists were averaged for each treatment to calculate the means. Parameter means demonstrating square cosines greater than 0.60 were considered for principal components; values close to 1 indicate that the parameter is well projected on the axes (Le, Josse, & Husson, 2008).

3. Results and discussion

3.1. Physico-chemical attributes of meat from deboned hams

The data on physico-chemical attributes of meat from fresh deboned hams are presented in Table 2. There were interactions ($P < 0.05$) between sex and dietary ractopamine on all the attributes; the results indicate that the effect of ractopamine is sex-specific.

Dietary ractopamine increased ($P<0.05$) the pH of meat from GT and IM, but not in SR ($P>0.05$). SR-RAC, SR-CON, and GT-RAC exhibited the greatest ($P<0.05$) pH values followed by GT-CON and IM-RAC; IM-CON had the lowest ($P<0.05$) pH value. Our findings on pH are in disagreement with those reported by Font i Furnols et al. (2012), who observed that semimembranosus muscle from immunocastrated Duroc barrows had greater pH than those from gilts. The differences in pH between the present study and Font i Furnols et al. (2012) can be attributed to the differences in the breed and muscle source (Monin, Mejenes-Quijano, Talmant, & Sellier, 1987).

Ractopamine did not influence ($P > 0.05$) water activity of meat. However, within CON diet groups, SR demonstrated greater ($P<0.05$) water activity than GT. There were no differences ($P>0.05$) between the groups within RAC diet indicating that dietary ractopamine nullified the variations in water activity. Ractopamine supplementation increased ($P<0.05$) the moisture content of meat only in IM, whereas it increased ($P<0.05$) the protein content in SR and IM. Lipid content of meat was decreased ($P<0.05$) by ractopamine in GT and IM.

It is well documented that ractopamine increases leanness in finishing pigs (Andretta et al., 2012; Boler et al., 2010; Carr et al., 2009; Kutzler et al., 2011; Rikard-Bell et al., 2009). The mode of action of beta-agonists is through modulation of calpain-dependent protein turnover (McDonagh, Fernandez, & Oddy, 1999; Wheeler & Koohmaraie, 1992) by increasing calpastatin expression. This is reflected in the greater protein content in meat from IM-RAC and SR-RAC than their CON counterparts. The high protein content in meat increases alkaline components, which can result in an increase in pH value. This could have partially contributed to the greater pH of IM-RAC than their control counterparts. In contrast to our results, several authors reported no difference in the ultimate meat pH between GT, SR, IM, and entire boars (Boler et al., 2010; Gispert et al., 2010; Pauly, Spring, O'Doherty, Kragten, & Bee, 2009; Zamaratskaia et al., 2008).

Ractopamine demonstrated a pronounced effect on IM animals; meat from these animals contained greater protein and moisture and lower lipid contents than their control counterparts. These results indicated that the negative effects of immunocastration, such as low moisture and high lipid contents can be mitigated by dietary ractopamine. While ractopamine decreased the lipid contents in meat from the gilts, this effect was not observed in SR. Our results suggest that ractopamine

Table 3
Effect of sex on physico-chemical parameters of pork frankfurters.

Parameters	Treatments[z]			SEM
	GT	SR	IM	
Cooking yield (%)	91.85[b]	91.90[b]	93.45[a]	0.34
pH	6.21[ab]	6.23[a]	6.18[b]	0.01
Protein (%)	12.7	12.54	12.58	0.10

SEM = standard error of the mean.
[a–b]Means in a row without common superscripts are different ($P<0.05$).
[z]GT = gilts; SR = surgically castrated male pigs; IM = immunocastrated male pigs.

Table 4
Effects of dietary ractopamine on physico-chemical parameters of pork frankfurters.

Parameters	Treatments[z]		SEM
	CON	RAC	
Cooking yield (%)	92.10	92.70	0.28
pH	6.17[b]	6.24[a]	0.01
Protein (%)	12.41[b]	12.80[a]	0.08

SEM = standard error of the mean.
[a–b]Means in a row without common superscripts are different ($P < 0.05$).
[z]CON = 0 ppm ractopamine diet; RAC = 7.5 ppm ractopamine diet.

is not effective in SR to improve meat proximate composition. Although ractopamine is a potent stimulator of adipose tissue lipid mobilization, it often does not reduce fat deposition because of rapid down-regulation of beta-adrenergic receptors, lack of effect on lipogenesis, and/or reduced sensitivity to beta-agonists (Rikard-Bell et al., 2009).

Our data are in agreement with previous research, which reported that ractopamine favors protein accretion (Carr et al., 2009; Dunshea, King, Campbell, Sainz, & Kim, 1993; Rikard-Bell et al., 2009). The effects of sex observed in the present study are in agreement with Carr et al. (2009), who reported that gilts had greater protein content in soft tissues than surgically castrated males. In contrast, Boler et al. (2010) did not observe any differences in the protein content of fresh hams from gilts and barrows.

3.2. Physico-chemical attributes of frankfurters

There was no interaction ($P > 0.05$) between sex and dietary ractopamine on cooking yield, pH, and protein content. While sex influenced ($P<0.05$) cooking yield and pH (Table 3), ractopamine influenced ($P<0.05$) pH and protein content (Table 4). Frankfurters from IM exhibited greater ($P < 0.05$) cooking yield than those from other sexes. SR demonstrated greater ($P < 0.05$) pH than IM. Ractopamine increased ($P<0.05$) both pH and protein content in frankfurters.

The data on frankfurter parameters influenced by the interaction ($P < 0.05$) between dietary ractopamine and sex are presented in Table 5. There was no effect ($P>0.05$) of RAC on water activity. Although SR-CON exhibited lower ($P < 0.05$) water activity than GT-CON, their RAC counterparts had similar ($P>0.05$) values. GT-RAC demonstrated the greatest ($P < 0.05$) water activity, while SR-CON had the lowest ($P< 0.05$). Dietary ractopamine increased ($P<0.05$) moisture content of frankfurters from GT and IM. Frankfurters from IM-RAC and GT-RAC showed greater ($P<0.05$) moisture contents than other groups; GT-CON had the lowest ($P < 0.05$) moisture content. Frankfurters manufactured from ractopamine fed GT and IM demonstrated lower ($P<0.05$) lipid content than their control counterparts reflecting their decreased lipid contents in meat (Table 2).

Emulsion-type meat products are a complex matrix, wherein water droplets, fat globules, and other ingredients are entrapped within a protein network. The variations in lipid, moisture, and protein content in fresh meat, due to sex and diet, can thus influence the quality of such products. To our knowledge, the present study is the first to evaluate the influence of immunocastration and dietary ractopamine on the quality attributes of emulsion-type pork products. The previous investigation on hams (Boler et al., 2010) reported no effect of sex, ractopamine feeding, or their interaction on cooking yield. Our data are in partial agreement with aforementioned authors as GT and SR as well as RAC and CON had similar results for cooking yield.

Table 6
Effect of dietary ractopamine and sex on instrumental color of pork frankfurters.

Parameters	Treatments[z]						SEM
	CON			RAC			
	GT	SR	IM	GT	SR	IM	
L^*	75.23[a]	73.69[c]	74.92[a]	73.87[bc]	74.69[a]	74.33[b]	0.13
a^*	4.41[bc]	4.66[ab]	5.05[a]	5.22[a]	4.12[c]	4.56[abc]	0.13
b^*	11.37[abcd]	11.93[ab]	10.64[d]	11.39[c]	11.2[bcd]	12.19[a]	0.18
Chroma	12.23[bc]	12.94[a]	11.79[c]	12.56[b]	12.3[bc]	12.7[ab]	0.13
Hue	65.69[bc]	68.75[b]	64.28[c]	65.18[c]	70.92[a]	70.42[a]	0.73

SEM = standard error of the mean.
[a–d]Means in a row without common superscripts are different ($P < 0.05$).
[z]CON = 0 ppm ractopamine diet; RAC = 7.5 ppm ractopamine diet; GT = gilts; SR = surgically castrated male pigs; IM = immunocastrated male pigs.

3.3. Instrumental color of frankfurters

Color parameters exhibited sex-specific response ($P < 0.05$) to RAC (Table 6). Dietary ractopamine decreased L^* values ($P<0.05$; promoted darker appearance) in frankfurters from GT and IM, while it increased L^* values ($P<0.05$; promoted lighter appearance) in SR. Ractopamine increased ($P<0.05$) a^* values (redness) in GT, while it decreased ($P<0.05$) redness in SR. IM-CON and GT-RAC exhibited greater ($P<0.05$) a^* values than GT-CON and SR-RAC. The b^* values (yellowness) of frankfurters from ractopamine fed IM were greater ($P<0.05$) than their counterparts in control diet. In addition, IM-RAC exhibited the greatest ($P < 0.05$) b^* value, whereas IM-CON had the lowest ($P < 0.05$) b^* value. While ractopamine increased ($P < 0.05$) chroma in IM, the reverse ($P < 0.05$) was observed in SR. On the other hand, RAC increased ($P < 0.05$) the hue values in SR and IM.

The range of data for color parameters in the present study is in agreement with those of Choe, Kim, Lee, Kim, and Kim (2013) in pork frankfurters. Pietrasik (1999) as well as Cengiz and Gokoglu (2007) reported that the color of frankfurter-type products is influenced by fat content, added water, and pigmentation of the raw meat used. Increased lipid content can decrease redness; this was observed in frankfurters manufactured from gilts, where GT-CON demonstrated lower a^* and greater lipid content than GT-RAC (Table 5). Non-meat ingredients are added to increase extractability of meat proteins and for improving emulsion stability. Furthermore, cooking-induced protein denaturation influences physico-chemical properties of emulsion (Totosaus, Montejano, Salazar, & Guerrero, 2002). Together, these changes could have modulated some of the observed effects of ractopamine and immunocastration on pork frankfurters.

Table 5
Effect of dietary ractopamine and sex on proximate composition of pork frankfurters.

Parameters	Treatments[z]						SEM
	CON			RAC			
	GT	SR	IM	GT	SR	IM	
a_w	0.9773[ab]	0.9737[c]	0.9760[abc]	0.9797[a]	0.9760[abc]	0.9743[bc]	0.0007
Moisture (%)	53.28[d]	53.94[c]	54.56[b]	56.68[a]	54.26[bc]	56.15[a]	0.11
Lipid (%)	29.50[a]	28.44[a]	28.68[a]	25.55[b]	28.28[a]	25.96[b]	0.21

SEM = standard error of the mean.
[a–d]Means in a row without common superscripts are different ($P < 0.05$).
[z]CON = 0 ppm ractopamine diet; RAC = 7.5 ppm ractopamine diet; GT = gilts; SR = surgically castrated male pigs; IM = immunocastrated male pigs.

Table 7
Effect of dietary ractopamine and sex on instrumental texture parameters of pork frankfurters.

Parameters	Treatments[z]						SEM
	CON			RAC			
	GT	SR	IM	GT	SR	IM	
Hardness (N)	20.50^{bc}	22.49^{ab}	17.66^{c}	18.25^{c}	21.39^{abc}	25.11^{a}	0.90
Springiness	0.81^{c}	0.83^{abc}	0.81^{c}	0.85^{ab}	0.82^{bc}	0.85^{a}	0.01
Cohesiveness	0.62^{a}	0.58^{ab}	0.52^{b}	0.52^{b}	0.58^{ab}	0.60^{ab}	0.02
Resistance	0.32^{a}	0.30^{ab}	0.25^{b}	0.27^{ab}	0.29^{ab}	0.32^{a}	0.01
Shear force (N)	6.57^{c}	10.00^{a}	6.08^{c}	9.02^{ab}	8.34^{b}	6.87^{c}	0.26

SEM = standard error of the mean.
[a–c]Means in a row without common superscripts are different ($P < 0.05$).
[z]CON = 0 ppm ractopamine diet; RAC = 7.5 ppm ractopamine diet; GT = gilts; SR = surgically castrated male pigs; IM = immunocastrated male pigs.

3.4. Instrumental texture of frankfurters

The instrumental texture parameters of frankfurters are presented in Table 7. There was interaction ($P<0.05$) between sex and diet. Dietary ractopamine increased ($P<0.05$) hardness of frankfurters manufactured from IM. While IM-RAC demonstrated greater ($P<0.05$) hardness than GT-RAC, SR-CON exhibited greater ($P < 0.05$) hardness than IM-CON. With respect to springiness, frankfurters from GT-RAC and IM-RAC animals exhibited greater ($P<0.05$) values than their control counterparts. Frankfurters from GT-CON demonstrated greater ($P < 0.05$) cohesiveness than GT-RAC. Ractopamine increased ($P<0.05$) resistance of frankfurters from IM. RAC did not influence ($P>0.05$) hardness, springiness, cohesiveness, and resistance of SR (Table 7). The effect of dietary ractopamine on frankfurter shear force was sex-specific ($P < 0.05$; Table 7). While RAC increased ($P<0.05$) shear force in GT, the reverse effect ($P < 0.05$) was observed in SR. There was no effect ($P > 0.05$) of RAC on shear force in IM. SR-CON demonstrated the greatest ($P<0.05$) shear force, whereas IM-CON, IM-RAC, and GT-CON had the lowest ($P<0.05$) values.

Although the present study was the first to report differences in textural attributes of frankfurters from ractopamine fed pigs, previous investigations (Athayde et al., 2012; Xiong et al., 2006) observed an increase in the shear force values of whole-muscle pork loins from ractopamine fed animals. It is not appropriate to extrapolate the observations from these studies to frankfurters because while manufacturing emulsions the meat is finely chopped and the muscle structure is disrupted.

3.5. Sensory evaluation

There was no interaction ($P > 0.05$) between sex and dietary ractopamine on the sensory attributes. The effect of ractopamine on sensory attributes is presented in Table 8; RAC frankfurters exhibited better ($P < 0.05$) texture than their CON counterparts. The improved texture for RAC frankfurters can be partially explained on the basis of their relatively high moisture and protein contents (Tables 4 and 5).

Table 8
Effect of dietary ractopamine on sensory attributes of pork frankfurters.

Attributes	Treatments[z]		SEM
	CON	RAC	
Appearance	6.52	6.69	0.13
Odor	6.79	6.99	0.12
Texture	6.69^{b}	7.07^{a}	0.13
Flavor	6.85	7.08	0.13
Overall acceptance	6.87	7.02	0.11
Purchase intention	3.62	3.79	0.09

SEM = standard error of the mean.
[a–b]Means in a row without common superscripts are different ($P<0.05$).
[z]CON = 0 ppm ractopamine diet; RAC = 7.5 ppm ractopamine diet.

Table 9
Effect of sex on sensory attributes of pork frankfurters.

Attributes	Treatments[z]			SEM
	GT	SR	IM	
Appearance	6.67	6.82	6.32	0.16
Odor	6.91	6.96	6.8	0.14
Texture	6.87	7.14	6.63	0.15
Flavor	6.89^{b}	7.38^{a}	6.63^{b}	0.16
Overall acceptance	6.97	6.76	7.11	0.14
Purchase intention	3.7^{ab}	3.97^{a}	3.44^{b}	0.11

SEM = standard error of the mean.
[a–b]Means in a row without common superscripts are different ($P<0.05$).
[z]GT = gilts; SR = surgically castrated male pigs; IM = immunocastrated male pigs.

The effect of sex on sensory attributes is presented in Table 9. Flavor and purchase intention were the only parameters influenced ($P<0.05$) by sex. Frankfurters from SR had greater ($P<0.05$) flavor scores than GT and IM. This could have contributed, in part, to the greater ($P<0.05$) purchase intention for frankfurters from SR than those from IM. On the other hand, the panelists rated the frankfurters from three sexes similar ($P>0.05$) in appearance, odor, and texture. Appearance is the first attribute considered by consumers in appraising product quality, followed by odor, texture, and then flavor (Meilgaard, Civille, & Carr, 2007). Based on this stepwise approach, the sensory data suggest that frankfurters from different sexes are rated similar up to the texture. The last perceived attribute (flavor) was the one that differentiated the treatments; SR received greater flavor scores than the other sexes, which could be partially explained based on the relatively high lipid content in SR frankfurters (Table 5).

3.6. Principal component analysis of frankfurter quality parameters

This multivariate analysis explained 77.43% of total variance in data (Fig. 1). Principal component 1 contributed to 47.10% of the variance and categorized the treatments into two groups; the first group consisted of GT-CON and IM-CON, whereas the second group was formed by SR-CON, SR-RAC, IM-RAC, and GT-RAC. This categorization was based on appearance, flavor, odor, texture, purchase intention, pH, hue, and chroma. Several parameters demonstrated strong correlation with others. The sensory texture was positively correlated to pH ($r = 0.925$) indicating that pH plays an important role in texture. Furthermore, purchase intention was positively correlated to appearance ($r = 0.972$), flavor ($r = 0.985$), and sensory texture ($r = 0.891$) scores, indicating the criticality of these parameters in consumers' purchase decisions. The second group (consisting of SR-CON, SR-RAC, IM-RAC, and GT-RAC) had greater sensory scores than the first group.

Principal component 2 explained the 30.33% of the total variance and categorized the treatments into two different groups, one consisting of SR-CON, GT-RAC and IM-RAC; and another one comprising SR-RAC, GT-CON and IM-CON. The parameters related to principal component 2 were a^* value, springiness, and moisture and lipid contents. While lipid content was negatively correlated to moisture content ($r = -0.977$) and springiness ($r = -0.955$), moisture content was positively correlated to springiness ($r = 0.879$). Frankfurters manufactured from SR-CON, GT-RAC and IM-RAC were rated better in springiness than those from SR-RAC, GT-CON and IM-CON, possibly due to differences in moisture and lipid contents (Table 5). The combination of the two principal components categorized the treatments into three distinct groups, i.e. SR-CON, GT-RAC and IM-RAC; GT-CON and IM-CON; and SR-RAC. This arrangement separated each RAC treatment from their respective CON counterpart. GT-CON and IM-CON were more distant from their RAC counterparts than SR-CON and SR-RAC (Fig. 1), indicating that dietary ractopamine influenced the quality parameters of frankfurters from GT and IM at a greater degree than those from SR.

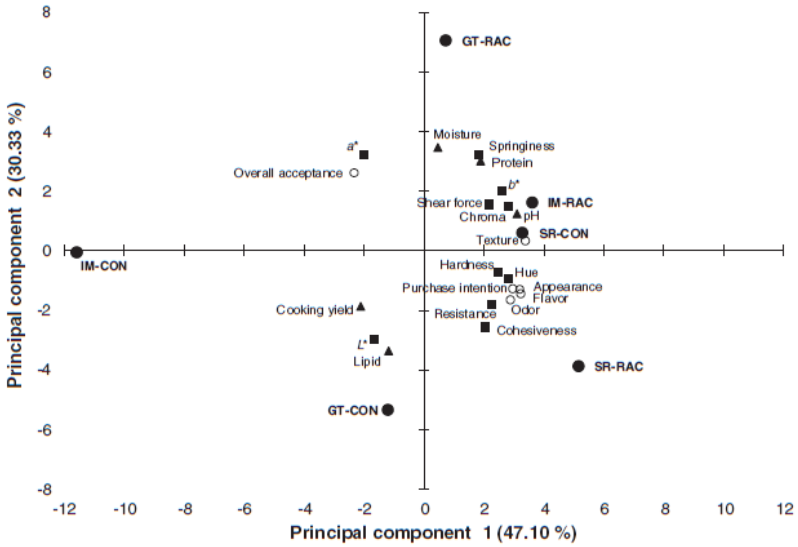

Fig. 1. Instrumental, sensory, and physico-chemical data of pork frankfurters in the plane defined by two principal components.

4. Conclusions

The results of the present study indicated that the effects of dietary ractopamine on the quality attributes of pork frankfurters were different in gilts, immunocastrated male pigs, and surgically castrated male pigs. Ractopamine demonstrated sex-specific effects on pork frankfurters and, to a limited extent, nullified the negative effects of immunocastration on the quality of pork frankfurters. Since more than three-fourths of pork is retailed and consumed as processed meats, swine industry may adopt sex-specific dietary strategies to improve pork quality.

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