NARA REGINA BRANDÃO CÔNSOLO

The use of β-adrenergic agonist in beef cattle diet

Pirassununga

2015

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Tese apresentada ao Programa de Pós-Graduação em Nutrição e Produção Animal da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo para a obtenção do título de Doutor em Ciências

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Prof. Dr. Prof. Dr. Luis Felipe Prada e Silva.

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Comissão de Ética no Uso de Animais

CERTIFICADO

Certificamos que o Projeto intitulado "Uso de agonistas β-adrenergicos na dieta de bovinos de corte", protocolado sob o nº 2474/2011, utilizando 72 (setenta e dois) bovinos, sob a responsabilidade do Prof. Dr. Luis Felipe Prada e SilvaMarcos Veiga dos Santos, está de acordo com os princípios éticos de experimentação animal da "Comissão de Ética no uso de animais" da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo e foi aprovado em reunião de 16/5/2011.

We certify that the Research "The use of β -adrenergic agonist in beef cattle diet", protocol number 2474/2011, utilizing 72 (seventy two) bovine, under the responsibility Prof. Dr. Luis Felipe Prada e Silva, agree with Ethical Principles in Animal Research adopted by "Ethic Committee in the use of animals" of the School of Veterinary Medicine and Animal Science of University of São Paulo and was approved in the meeting of day 05/16/2011.

São Paulo, 19 de outubro de 2015.

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DEDICATÓRIA

Dedico esse estudo, todo meu trabalho e esforço para as pessoas que mais amo nessa vida, pois sem eles nada disso e nada de mim seria possível.

Ao meu grande Pai Tarcísio Cônsolo
À minha forte Mãe Fátima Maria Brandão Cônsolo
À minha queridíssima Irmã Lara Cristina Brandão Cônsolo

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“Se ficar saudade
Deixa que mais tarde ela também vai
Como vai a vida
Vou tocando em frente sem olhar pra trás

Coisas que acontecem
Deixa que eu lhe diga, nada me cansou
Não senti fadiga
Porque gente amiga só me traz calor

Se eu tô indo embora
Não vai ser agora que se vai sofrer
Deixa que mais tarde
Na curva da estrada a gente se vê

Se eu deixo saudade
Vou levar também
Ter que ir embora
Todo mundo tem”

Almir Sater e Renato Teixeira

RESUMO

CÔNSOLO, N. R. B. **Uso de agonista β-adrenérgico na dieta de bovinos de corte**. 2015. 85 f. Tese (Doutorado em Ciências) – Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, 2015.

O objetivo desse estudo foi avaliar o efeito do Cloridrato de Zilpaterol (ZH), Ractopamina (RH) e aumento no nível de proteína da dieta, em dois experimentos distintos na dieta de animais Nelore em sistema de confinamento, nas características produtivas, metabólitos sanguíneos, características de carcaça, composição de corpo vazio e ganho, qualidade da carne e expressão gênica. Para isso, foram realizados dois experimentos. O Exp. 1 utilizou 48 machos, não castrados, distribuídos em blocos casualisados em 4 tratamentos em arranjo fatorial 2x2, sendo dois níveis de PB: 100 e 120% e dois níveis de RH: 0 e 300mg/animal/dia. No Exp. 2, foram utilizadas 72 novilhas da raça Nelore distribuídas em delineamento em blocos casualizados. As novilhas foram separadas em dois grupos: Controle (CONT) e Zilpaterol (ZH). Em ambos os experimentos, os animais foram pesados periodicamente e amostras de sangue foram coletadas. Após o abate, o PCQ foi avaliado, amostras da musculatura foram obtidas para análise de PCR. Depois do resfriamento das carcaças, a AOL e EGS foram avaliados, amostras do músculo *Longissimus* e a sessão entre a 9ª e 11ª costelas foram coletadas. Além disso, no Exp. 2, as novilhas foram abatidas de forma seriada, 0, 20 e 30 dias de suplementação com ZH e no último abate, foi realizada a desossa completa da meia carcaça. A suplementação com RH aumentou o GMD, diminuiu CMS na dieta com PB120, e aumentou a eficiência alimentar, o teor de proteína plasmática e a atividade da ALP na dieta PB120, diminuiu a glicose na dieta PB100. Além disso, a RH diminuiu a FC nas carnes sem maturação e a dieta com 120% de proteína aumentou a FC nas carnes 0 e 7 dias maturadas. No Exp. 2, as novilhas suplementadas com ZH tiveram aumento no GMD, PCQ, EA, creatinina plasmática e diminuição dos AGNE. A composição de corpo vazio foi alterada após 20 dias de suplementação com ZH. O ZH aumentou o ganho de PCV, ganho de proteína e rendimento da maioria dos cortes do traseiro especial. Houve um aumento na FC aos dias 7 e 14 de maturação. O painel sensorial detectou diminuição da maciez da carne de animais alimentados com ZH, independentemente do tempo de maturação, e diminuição a suculência da carne nos tempos 0 e 14 de maturação. O ZH aumentou a expressão das enzimas calpaínas e calpastatina. Os resultados do

presente estudo mostram aumento no desempenho produtivo dos animais alimentados com RH e ZH. A suplementação com ZH aumentou o rendimento dos cortes e mudou a composição corporal de ganho. O uso de agonista β-adrenérgico melhora a eficiência do sistema e aumenta a deposição de tecido magro da carcaça, gerando maior rentabilidade por animal abatido.

Palavras-chave: Eficiência alimentar. Qualidade da carne. Nelore. Ractopamina. Zilpaterol.

ABSTRACT

CÔNSOLO, N. R. B. **The use of β-adrenergic agonist in beef cattle diet.** [Uso de agonista β-adrenérgico na dieta de bovinos de corte]. 2015. 85 f. Tese (Doutorado em Ciências) – Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, 2015.

The aim of this study was to evaluate the use of Zilpaterol and Ractopamine Hydrochloride, in two distinct experiments on Nellore diet in feedlot system, for growth performance, serum metabolites, carcass traits, empty body weight composition, carcass gain composition, meat quality and gene expression. The Exp. 1 used 48 bulls, randomly assigned to four treatments in a 2x2 factorial arrangement; two levels of dietary CP: 100% and 120% of metabolizable protein requirement, defined as CP100 and CP120, respectively. Moreover, two levels of RH: 0 and 300 mg/animal/per day. The Exp. 2 used 72 Nellore heifers assigned to a randomized block design. Heifers were separated into 2 groups: Control and Zilpaterol (ZH). In both experiments, the animals were weighed and blood samples were taken periodically. Animals were slaughtered and HCW was recorded, muscle samples were taken to PCR analysis. After chilling, LMA and fat thickness were recorded, longissimus samples and the 9–11th rib section were obtained. In addition, in the Exp. 2, heifers were sub groups were slaughterer at 0, 20 and 30 days of ZH supplementation, in the last slaughterer, the carcass were debone. Supplementation with RH increased ADG, reduced DMI at CP120, improved G:F, plasma total protein at CP120, also decreased plasma glucose concentration at CP100, and increased ALP activity at CP120. Ractopamine decreased meat shear force, at day 0 of aging. Greater dietary protein increased meat shear force after 0 and 7 days of aging. Heifers fed ZH had gains in ADG, HCW, G:F ratio, increased serum creatinine, and decreased serum NEFA. Zilpaterol increased carcass dressing percentages, and also decreased kidney-pelvic fat. The EBW composition was changed after 20 d of ZH supplementation. The ZH increased EBW gain, EB protein gain, and subprimal yield. There was an increase on WBSF after 7 and 14 d of aging. The sensory panelists reported a decreased on meat tenderness by ZH supplementation, regarding aging time, and a decrease on juciness at 0 and 14 d of aging. The ZH increased the calpain and calpastatin gene expression. These results indicate the efficiency of RAC and ZH to improve the performance,feed efficiency, and muscle mass deposition in Nellore bulls and heifers. Greater CP did not further

improved the RAC effect. The ZH supplementation increased the subprimal yield and changed body gain composition. Finally, the use of β-adrenergic agonist increase the beef cattle system´s efficiency by increasing the lean carcass component, with greater profitability per slaughtered animal.

Keywords: Feed efficiency. Meat quality. Nellore. Ractopamine. Zilpaterol.

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Summary

1 INTRODUCTION

Brazilian beef cattle production represents a substantial portion of GDP in Brazil, generates numerous direct and indirect jobs and may be one of the only sectors with significant growth in the country, representing great part of the profitability in exports, and large responsibility for the trade surplus of the country (CEPEA, 2015).

Brazil has the largest commercial herd in the world, around 208.3 million heads spread over 2.6 million farms (ABIEC, 2014; CEPEA, 2011) and, since 2004, has consolidated the role of major world beef exporter. Indeed, it currently exports to over 80 countries, gradually conquering the world market (ABIEC, 2015). Today the country ranks third position in world beef consumption and it is the second in equivalent carcass production, about 10.07 thousand tons (ABIEC, 2014).

From the nineties, with the constraints of globalization, the livestock sector (beef cattle) has shown significant advances in development, which has been increasing its productive and economic competitiveness. Formerly, the growth of Brazilian beef cattle was due by the incorporation of new lands, increasing the production area. However, these days, the focus is to increase system productivity. Moreover, to maintain the competitiveness of the national beef cattle in internal and international markets implies in meat production with maximum efficiency and with a standard of quality, which meets the most demanding markets. In order to bring this aspect to the Brazilian beef cattle, the use of β-adrenergic agonist has been used. The β-adrenergic agonists are organic molecules added to the finishing diet of beef cattle in feedlot system. This product, when ingested, passes unaltered through the rumen and is absorbed in the small intestine. Reaching the bloodstream, these molecules attain the “target cells”.

Accessing the "target cells", the β-adrenergic agonists bind to specific membrane receptors, β1 and β2 type. These, in turn, are coupled to G protein, which

is activated in the presence of β-adrenergic agonist, causing extracellular signals to be converted into intracellular signals. The G-protein then activates adenylyl cyclase, the enzyme that produces cyclic adenosine monophosphate (cAMP), one of the major intracellular signaling molecules (MERSMANN, 1998). The mechanism by which cAMP produces effects is to bind to the regulatory subunit of protein kinase A to release the catalytic subunit that then phosphorylates a number of intracellular proteins. Some of these proteins are enzymes that are activated when phosphorylated (e.g., hormone sensitive lipase, the rate-limiting enzyme for adipocyte triacylglycerol degradation; calpastatin responsible by decrease muscle degradation; MERSMANN, 1998). The cAMP response element binding protein (CREB) is phosphorylated by protein kinase A; the CREB binds to a cAMP response element in the regulatory part of a gene and stimulates the transcription of that gene. Phosphorylation increases the transcriptional activity of the CREB, providing the mechanism for β-adrenergic agonist mediated transcription of a number of genes in the mammalian cell (MERSMANN, 1998). Other enzymes become inactivated when phosphorylated (e.g., acetyl-CoA carboxylase, the rate-limiting enzyme for long-chain fatty acid biosynthesis; μ-calpain- and m-calpain, responsible by muscle degradation; MERSMANN, 1989).

After all, the main effects of beef cattle fed with β-adrenergic agonists are muscle hypertrophy, with improve on performance, G:F ration, LMA, subprimal cuts yield and reduction in adipose tissue deposition, being the Zilpaterol and the Ractopamine Hydrochloride the most used ones. The effect of these products is well known in *Bos taurus* breeds, however the action of the product for *Bos indicus* is hardly reported. Thus, the aim of this study was to evaluate the use of Zilpaterol and Ractopamine Hydrochloride, in two distinct experiments on Nellore diet in feedlot system, for growth performance, serum metabolites, carcass traits, empty body weight

composition, carcass gain composition, meat quality and gene expression of enzymes related to the deposition of muscle tissue.

2 CHAPTER 1 / PAPER 1

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Effects of ractopamine hydrochloride and dietary protein content on performance, carcass traits and meat quality of Nellore bulls

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2.1 ABSTRACT

Ractopamine hydrochloride (RH) alters protein metabolism and improves growth performance in Bos taurus cattle with high carcass fat. Our objective was to evaluate the effects of RH, dietary CP, and RH x CP interaction on performance, blood metabolites, carcass characteristics, and meat quality of young Nellore bulls. Forty eight bulls were randomly assigned to 4 treatments in a 2x2 factorial arrangement. The factors were two levels of dietary CP (100 and 120% of MP requirement, defined as CP100 and CP120, respectively), and two levels of RH (0 and 300 mg·animal-1·d-1). Treated animal received RH for the final 35 d before slaughter. Animals were weighed at the beginning of the feedlot period (d -63), at the beginning of ractopamine supplementation (d 0), after 18 d of supplementation (d 18), and before slaughter (d 34). Animals were slaughtered and hot carcass weights recorded. After chilling, carcass data was collected and longissimus samples were obtained for determination of meat quality. The 9–10–11th rib section was removed for carcass composition analysis. Supplementation with RH increased ADG independently of dietary CP. There was a RH x CP interaction on DMI, where RH reduced DMI at CP120, with no effect at CP100. Ractopamine improved feed efficiency, without RH x CP interaction. Ractopamine had no effect on plasma creatinine and urea concentration. Greater dietary CP tended to increase blood urea, and there was a RH x CP interaction for plasma total protein. Ractopamine supplementation increased plasma total protein at CP120, and had no effect at CP100. Ractopamine also decreased plasma glucose concentration at CP100, but had no effect at CP120. Ractopamine increased ALP activity at CP120 and had no effect at CP100. There was a tendency for RH to increase longissimus muscle area, independently of dietary CP. Ractopamine did not alter fat thickness; however, fat thickness was reduced by greater CP in the diet. Supplementation with RH decreased meat shear force, but only at day 0 of aging,

having no effect after 7, 14 or 21 d. Greater dietary protein increased meat shear force after 0 and 7 d of aging, with no effect after 14 or 21 d. These results demonstrate for the first time the efficacy of ractopamine supplementation to improve gain and feed efficiency of intact Bos indicus males, with relatively low carcass fat content. Ractopamine effects were not further improved by increasing dietary protein content above requirements.

Key words: Bos indicus, Meat tenderness, Muscle, Protein, Ractopamine

2.2 INTRODUCTION

Efficient use of nutrients is vital for profitability and sustainability of beef cattle production. Beta-adrenergic agonists (**β-AA**) are additives commonly used to improve the efficiency of gain in the USA beef industry. In December 2011 the use of ractopamine in feedlot cattle was approved in Brazil. The efficacy of β-AA has been demonstrated in several studies with mostly young, castrated, *Bos taurus* breeds, with a high degree of fat thickness, and marbling (GRUBER et al., 2007; QUINN et al., 2008; SCRAMLIN et al., 2010). However, the efficacy of β-AA has yet to be demonstrated in *Bos indicus* intact bulls, with reduced fat thickness (slaughter at 20% carcass fat), a common situation in Brazilian herds.

Ractopamine is a β1-Adrenergic agonist that promotes the repartition of nutrient flow away from lipogenesis, and towards protein accretion (YANG and MCELLIGOTT, 1989). In general, feeding ractopamine increases average daily gain, improves feed efficiency, and increases both live and hot carcass weight (SCHROEDER, 2004; DUNSHEA et al., 2005; AVEDAÑO et al., 2006). This increase in muscle mass is attributed to an increase in muscle protein synthesis, a reduction in protein

degradation, or some combination of both (SCRAMLIN et al., 2010). Additionally, other beta-adrenergic agonists increase the transcriptional activity of calpastatin (RATHMANN et al., 2009), which could be the reason for the reduction in tenderness that has been reported in some studies (LEHESKA et al., 2008; KELLERMEIER et al., 2009).

Based on studies with other species, rapid increase in fractional rate of muscle protein synthesis occurs with oral administration of ractopamine, without reduction of fractional protein degradation rates (BEERMAN, 2002). Because of the increase on muscle accretion, protein requirements may also be enhanced in ractopamine treated animals. There are few data reporting the effect of dietary CP on cattle or swine fed with RH (MITCHELL et al., 1991; WALKER et al., 2006), and these reported no benefit in improving dietary CP in RH fed animals.

Therefore, the objective of this study was to evaluate effects of ractopamine and dietary CP content on feedlot performance, blood metabolites, carcass characteristics, and meat quality of Nellore bulls.

2.3 MATERIALS E METHODS

All animal procedures used in this study were conducted in accordance with the Institutional Animal Care and Use Committee Guidelines of the University of São Paulo, and approved by the FMVZ animal ethics committee (Protocol Number 2474-2011).

2.3.1 Experimental Site

The study was conducted at the Ouro Fino Experimental Station, located at the city of Guatapará, state of São Paulo, in southeast Brazil (21º29'48" S, 48º02'16" W, and 512 m above sea level) from September 2012 to January 2013. The animals were

in the feedlot for a total of 112 d, with the first 15 d for acclimatization of the animals to the facility and diets. Pen dimensions were 5 x 10 m with 2 m of concrete floor, and shade in the east-west orientation. Automatic water troughs, with float-activated water supplies, were located at the end of each pen. The concrete feed bunk in each pen was 3 m long, and pens were situated in one line.

2.3.2 Animals management and Diets

Forty-eight Nellore bulls, 24-26 months old, with average initial BW of 397 ± 15 kg, were randomly allotted to 16 pens, each containing 3 bulls. Four treatments were randomly assigned to the pens, in a 2 x 2 factorial arrangement of treatments. The factors were two levels of CP in the diet (100 and 120% of the MP requirement, defined as **CP100** and **CP120**, respectively), and two levels (0 and 300 mg·animal^{-1}·d^{-1}) of ractopamine hydrochloride (RH; Ouro Fino Animal Health, Cravinhos, SP, Brazil). The animals were kept on the experimental diets for a total of 97 d, and treated animals received RH for the final 35 d before slaughter.

Animals were fed twice daily a diet with 25:75 forage:concentrate ratio on a dry matter (**DM**) basis to provide 27% of NDF on the diet (Table 1). Chopped Tifton-85 hay was used as the roughage source. Ractopamine was added to the mineral supplements that were mixed daily into the total diet to provide a daily intake of 300 mg of RH to each animal. Diets were offered ad libitum, dry matter intake was measured daily by weighing of offered feed and orts weighing, and amount offered was adjusted daily allowing for a minimum of 5% orts during the experiment. In addition, ingredients and orts were sampled weekly and pooled to determine chemical composition. The animals remained in the feedlot system during 63 days, all fed the same diet, prior to the ractopamine supplementation period.

Table 1 - Composition and analysed nutrient content (DM basis) of the finishing bulls diets

Item[1]	Diets[2]	
	CP 100	CP 120
Ingredients		
Hay Tifton-85	25.0	25.0
Ground corn	63.7	62.3
Soybean meal	4.1	8.9
Urea	0.3	0.5
Mineral mixture	3.3	3.3
Analysed composition		
CP	12.5	14.7
NDF	27.0	27.0
TDN[3]	77.4	77.4
NE_g, MJ/kg DM	5.0	5.0

[1]The trace mineral mixture contained (per kg): Zinc 728mg, Iron 221mg, CP (min.) 109%, Fluorine (max.) 106mg, Calcium 116g, Selenium 3mg, Phosphorus 14g, Manganese 226mg, Copper 221mg, Cobalt 29mg, Iodine 21mg, Sodium 44g, Sulfur 43g, Potassium 47g, NNP - Equiv. Protein (max.) 109%, Monensin Sodium 1.000mg/kg; NE_g, MJ/kg DM
[2]Levels of CP in the diet (100 and 120% of the metabolizable protein requirement).
[3]Estimated according to National Research Council (NRC, 2000).
Fonte: Cônsolo, 2015.

To measure the performance, the animals were weighed at the beginning of the ractopamine supplementation (d 0), after 18 d of supplementation (d 18), and before slaughter (d 34). Animals were weighed after 18 hours of feed and water restriction. Average daily gain was calculated for the two periods: from d 0 to d 18 and from d 18 to d 34. Feed efficiency was calculated from average dairy gain (**ADG**) and dry matter intake (**DMI**) during the RH supplementation period (from d 0 to d 34).

2.3.3 Feed samples and chemical analyzes

Feed samples were taken during the morning feeding, and frozen for subsequent analysis of DM, organic matter (**OM**), ash, ether extract (**EE**), and CP, according to the methods described by the Association of Official Analytical Chemists (AOAC, 2000). Neutral detergent fibre was determined as in procedure B of Van Soest et al. (1991) using 8 M urea and heat stable α-amylase (Sigma Chemical Co., St. Louis, MO, USA), with no addition of sodium sulphite to avoid undesirable solubilisation of fibrous

compounds, such as lignin, in an ANKOM A200 Fibre Analyser (ANKOM® Technology, Fairpoint, NY, USA). Acid detergent lignin and ADF were analysed according to Van Soest and Robertson (1985).

2.3.4 Blood Sample collection and analysis

Blood was collected by venipuncture or puncture of the coccygeal artery, before the morning feeding, at the beginning and at the end of RH supplementation. Blood samples (10 mL) were collected into 10 mL BD Vacutainers® (BD Vacutainer, São Paulo, SP, Brazil), without anticoagulant, for the measurement of serum glucose, total protein, albumin, plasma urea nitrogen (**PUN**), aspartate aminotransferase (**AST**), γ-glutamyl transferase (**GGT**), and alkaline phosphatase (**ALP**). Blood parameters were assayed colorimetrically according to standard procedures using commercially available diagnostic kits (Laborlab®, São Paulo, SP, Brazil and CELM, São Paulo, SP, Brazil) in an ABS-200 Automatic Biochemistry Analyzer (CELM®, São Caetano do Sul, SP, Brazil).

2.3.5 Carcass data

On d 113, bulls were transported approximately 50 km to a commercial slaughter house and humanely slaughtered. Hot carcass weights were recorded at slaughter. After 24 h of chilling, longissimus muscle area (**LMA**) and fat thickness were measured between the 12th and 13th ribs, across the *longissimus dorsi*, at the left half-carcasses. All remaining meat quality measurements were carried out on cross-sectional samples of longissimus muscle. Instrumental colour readings were collected using a CR 200b Minolta colorimeter (Minolta Camera Co., Ltd., Osaka, OSA, Japan). Three scans were taken at the time of carcass fabrication, during five seconds each, and averaged to determine instrumental colour values. *Longissimus* samples were allowed to

oxygenate for 30 min before scanning. Results were expressed as L* (lightness), a* (redness) and b* (yellowness) in the CIELAB system, with the D65 light source. The a* value is a measure of a colour continuum from red to green, and b* value is a measure of a colour continuum from yellow to blue. Greater L* value denotes lighter meat and greater a* and b* values indicate a more red and yellow color, respectively.

For determination of Warner-Bratzler shear force analysis (**WBSF**), the four most anterior *longissimus dorsi* steaks were randomly assigned to each animal of four postmortem aging periods (0, 7, 14, and 21 d). Steaks were vacuum packaged and stored at 3°C. After completion of the appropriate aging time, steaks were frozen and stored at −20°C for subsequent WBSF measurements.

2.3.6 9–10–11th Rib Composition

The procedure developed by Hankins and Howe (1946) was used to mark the 9–10–11th rib section (**HH section**) on the left side of each carcass. At 24 h postmortem, carcasses were fabricated and the 9–10–11th rib section was removed from the primal rib. The samples were frozen, cut with chainsaw and ground in a large grinder (Frigmann Herman, Itupeva, SP, Brazil). Grounded samples were freeze-dried to constant weight with a lyophilizer (Itasul Import and Instrumental Technical Ltda, Porto Alegre, RS, Brazil), thus obtaining the water content of the HH samples.

Chemical composition of HH section was estimated according to Lanna et al. (1995). The water content of the 9–10–11th rib section was used to estimate the water and EE in the empty body weighed (**EBW**) using the following equations:

$$\% \text{ water EBW} = 24.1936 + (0.6574 \text{ X } \% \text{ water } 9\text{–}10\text{–}11^{th} \text{ rib section})$$

$$\% \text{ EE EBW} = 60.815 - (0.7968 \text{ X } \% \text{ water } 9\text{–}10\text{–}11^{th} \text{ rib section})$$

The protein and ash contents of EBW were estimated using 0.3009 as the protein:water ratio, and 0.0747 as the ash:water ratio (LEME et al., 1994).

2.3.7 Shear force and cooking loss analysis

Shear force (**SF**) and cooking loss (**CL**) were determined at the Laboratory the Laboratory the Animal Evaluation and Meat Quality of the University of São Paulo, using the methodology proposed by WHEELER et al. (2005) and previously described by CÔNSOLO et al. (2015). The steaks were thawed for 24 h at 4°C, weighed, and roasted in an oven equipped with a thermostat adjusted to 170°C (Flexa de Ouro Industry, São Paulo, SP, Brazil). The steaks internal temperature was monitored using individual thermometers (Globo Industry, Americana, SP, Brazil) inserted at the centre of the steak, until it reached 71°C. The steaks were cooled to 28°C and weighed again, thus obtaining the value for CL. Steaks were cooled at 4°C for 24 h before shearing. For WBSF evaluation, six cores with 1.3 cm of diameter were taken from each steak, parallel to the orientation of the muscle fibres (Ferrari furadeira, São Paulo, SP, Brazil). Each core was sheared perpendicular to the muscle fibre using a WBSF instrument (Warner-Bratzler meat Shear, G-R Manufacturing, Collins, KS, USA), according to standard procedures (American Meat Science Association, 1995). The WBSF values of the six subsamples were averaged for statistical analysis.

2.3.8 Statistical analysis

All statistical analyses were conducted using SAS version 9.1.2 for Windows (SAS Institute Inc., Cary, NC). Data were analysed as a completely randomized design with a 2 x 2 factorial arrangement of treatments, using the MIXED procedure. The model included the fixed effects of CP level (100 or 120% of requirements), the RH level (0 or 300 mg·animal^{-1}·day^{-1}), and their interaction. Pens were considered as the

experimental units (random effect). Data for ADG was analysed as a repeated measure for periods (two periods: from d0 to d18 and from d18 to d34). The model included the fixed effects of CP, RH, and their interaction, as well as the random effect of pen. Denominator degrees of freedom were calculated using the Kenward-Roger approximation. Meat quality data was also analysed as repeated measure for aging time, and the fixed effects of aging time and the interaction of aging time with treatments were included in the model.

Various error covariance structures were investigated and the one that best fit the data, according to the Bayesian information criterion (BIC), was selected. When there was a significant interaction, the effects of treatments were compared using the SLICE option of the MIXED procedure. Significance was declared at $P \leq 0.05$, and trends were considered at $P > 0.05$ and $P \leq 0.10$ for all analyses.

2.4 RESULTS

2.4.1 Performance

Body weight at the beginning of ractopamine supplementation (d 0) was similar for all treatments (P>0.10), demonstrating homogeneity at the initial allocation (Table 2). There was no RH x CP interaction on final BW (P=0.26), ADG (P=0.43), or on gain:feed ratio (**G:F**; P=0.78). Despite the numerical difference, there was no effect of RH supplementation on final body BW (P=0.14). On the other hand, animals supplemented with RH had 20% greater ADG than control animals (1.50 vs. 1.25 kg/d, P=0.03). Increasing dietary CP content above requirements had no effect on final BW, ADG or G:F ratio (P>0.05).

For DMI, the RH x CP interaction was significant (P<0.01). The decomposition of the interaction demonstrated that for DMI expressed as a percentage of BW, RH

supplementation had no effect on DMI at CP100 (*P*=0.12), yet it reduced DMI at CP120 (1.95 vs. 1.81 % BW for RH0 and RH300, respectively; *P*=0.03). Addition of RH to the diet considerably improved G:F ratio, independently of the CP concentration of the diet (0.15 vs. 0.13, *P*=0.02).

Table 2 - Effects of RH[1] and dietary protein content on live performance

Trait	CP 100[2]		CP 120[3]		SEM	*P*-Value		
	RH0	RH300	RH0	RH300		RH	CP	RHxCP[4]
Initial BW[5], kg	478	499	486	486	12	0.21	0.80	0.21
Final BW, kg	520	552	531	536	11	0.14	0.80	0.26
ADG, kg	1.20	1.54	1.30	1.46	0.11	0.03	0.85	0.43
DMI, kg/d	9.59^{b}	10.61^{a}	9.95^{ab}	9.25^{b}	0.30	0.58	0.09	<0.01
DMI, %BW	1.91^{ab}	2.01^{a}	1.95^{a}	1.81^{b}	0.07	0.66	0.07	0.01
G:F	0.13	0.14	0.13	0.15	0.01	0.02	0.51	0.78

[1]RH = ractopamine hydrochloride (Ouro Fino Saúde Animal, Cravinhos, SP, Brazil) fed at 0 or 300 $mg \cdot animal^{-1} \cdot d^{-1}$ during 35 d before slaughter
[2]Diet formulated to meet 100% of metabolizable protein requirements.
[3]Diet formulated to meet 120% of metabolizable protein requirements.
[4]Decomposition of the RH*CP interaction: within a row, means without a common superscript differ (*P*<0.05).
[5]Corresponds to the beginning of RH supplementation.
Fonte: Cônsolo, 2015

2.4.2 Blood samples

There was no RH x CP interaction on plasma creatinine (*P*=0.68) or urea concentration (*P*=0.43). There was also no effect of RH or dietary CP on plasma creatinine concentration (*P*>0.10, Table 3). Dietary CP level tended to increase blood urea (31.2 vs. 40.8 mg/dL for CP100 and CP120, respectively, *P*=0.07), with no effect of RH (*P*=0.28). There was an RH x CP interaction for plasma total protein concentration (*P*=0.02). Ractopamine supplementation increased plasma total protein at CP 120 (*P*=0.03) and had no effect at CP100 (*P*=0.17). There was also a significant RH x CP interaction for plasma glucose (*P*=0.04). Ractopamine supplementation decreased plasma glucose concentration at CP100 (*P*=0.05), and had no effect at CP120 (*P*=0.87). Regarding the plasma activity of the liver enzymes, there was no effect of RH x CP interaction for AST (*P*=0.64) or GGT (*P*=0.74). There was also no

effect of RH on the activities of these two liver enzymes (*P*>0.30). There was a tendency for greater AST activity at CP120 than at CP100 (200 vs 116 U/L, *P*=0.09), with no effect of CP on GGT activity (*P*=0.28). The RH x CP interaction was significant for ALP activity (*P*=0.05), as ractopamine supplementation increased ALP activity at CP120 (*P*=0.05), and had no effect at CP100 (*P*=0.42).

Table 3 - Effects of RH[1] and dietary protein content on plasma composition

Traits	CP 100[2]		CP 120[3]		SEM	*P*-Value		
	RH0	RH300	RH0	RH300		RH	CP	RHxCP[4]
Creatinine, mg/dl	1.88	1.98	1.64	1.90	0.19	0.36	0.41	0.68
Urea, mg/dl	32	30	45	36	5	0.28	0.07	0.43
Protein, mg/dl	3.78[b]	3.54[b]	3.72[b]	4.14[a]	0.13	0.49	0.05	0.02
Glucose, mg/dl	133[a]	97[b]	107[b]	108[b]	8	0.05	0.33	0.04
AST[5], U/L	120	114	180	220	46	0.73	0.09	0.64
GGT[5], U/L	22	26	27	35	7	0.32	0.28	0.74
ALP[5], U/L	406[a]	363[ab]	271[b]	385[a]	37	0.35	0.14	0.05

[1]RH = ractopamine hydrochloride (Ouro Fino Saúde Animal, Cravinhos, SP, Brazil) fed at 0 or 300 mg·animal^{-1}·d^{-1} during 35 d before slaughter.
[2]Diet formulated to meet 100% of metabolizable protein requirements.
[3]Diet formulated to meet 120% of metabolizable protein requirements.
[4]Decomposition of the RH*CP interaction: within a row, means without a common superscript differ (*P*<0.05).
[5]AST: aspartate aminotransferase; GGT: gamma glutamyltransferase; ALP: alkaline phosphatase.
Fonte: Cônsolo, 2015

2.4.3 Carcass traits and Meat quality

There were no RH x CP interaction effects on carcass traits or on longissimus colour (*P*>0.10). There was also no effect of RH or of CP on hot carcass weight (**HCW**) or dressing percentage of the carcass (*P*>0.10, Table 4). There was a tendency for RH supplementation to increase LMA (83.2 vs. 87.9 cm^2, *P*=0.07), with no effect of dietary CP (*P*=0.81). Ractopamine did not alter fat thickness (*P*=0.29); however, increasing dietary CP above requirements (CP120) decreased fat thickness (5.1 vs. 4.3 mm for CP100 and CP120, respectively, *P*=0.05).

Table 4 - Effects of RH[1] and dietary protein content on carcass traits and meat quality

Trait	CP 100[2]		CP 120[3]		SEM	*P*-Value		
	RH0	RH300	RH0	RH300		RH	CP	RHxCP[4]
HCW[4], kg	294	313	299	302	6	0.16	0.66	0.25
Dressing, %	56.5	56.6	56.3	57.1	0.4	0.41	0.77	0.49
LMA[5], cm^2	81.1	89.4	85.3	86.4	1.7	0.07	0.81	0.15
Fat thickness, mm	4.7	5.6	4.3	4.2	0.3	0.29	0.05	0.26
Color[6]								
*L**	33.2	32	34.5	31.6	1.2	0.13	0.74	0.48
*a**	17.9	17.9	19.2	17.6	0.9	0.30	0.81	0.29
*b**	10.9	9.6	15.1	15.5	0.7	0.56	<0.01	0.22

[1]RH = ractopamine hydrochloride (Ouro Fino Saúde Animal, Cravinhos, SP, Brazil) fed at 0 or 300 $mg \cdot animal^{-1} \cdot d^{-1}$ during 35 d before slaughter.
[2]Diet formulated to meet 100% of metabolizable protein requirements.
[3]Diet formulated to meet 120% of metabolizable protein requirements.
[4]HCW: hot carcass weight.
[5]LMA: Longissimus muscle area.
[6]*L** = Lightness, from 0 (black) to 100 (white); *a** = redness, from -120 (green) to 120 (red); *b**=yellowness, from -120 (blue) to 120 (yellow).
Fonte: Cônsolo, 2015

Meat colour scores were not affected by RH supplementation, and values were within the accepted normal range (MUCHENJE et al., 2009). Dietary CP altered the b* value (*P*<0.01), being greater for CP120 than CP100 (15.3 vs. 10.2 for CP120 and CP100, respectively). When analysing the chemical composition of the carcasses, as estimated by the Hankins and Howe (1946) method, treatments had no effect on the protein, fat or ash content (Table 5).

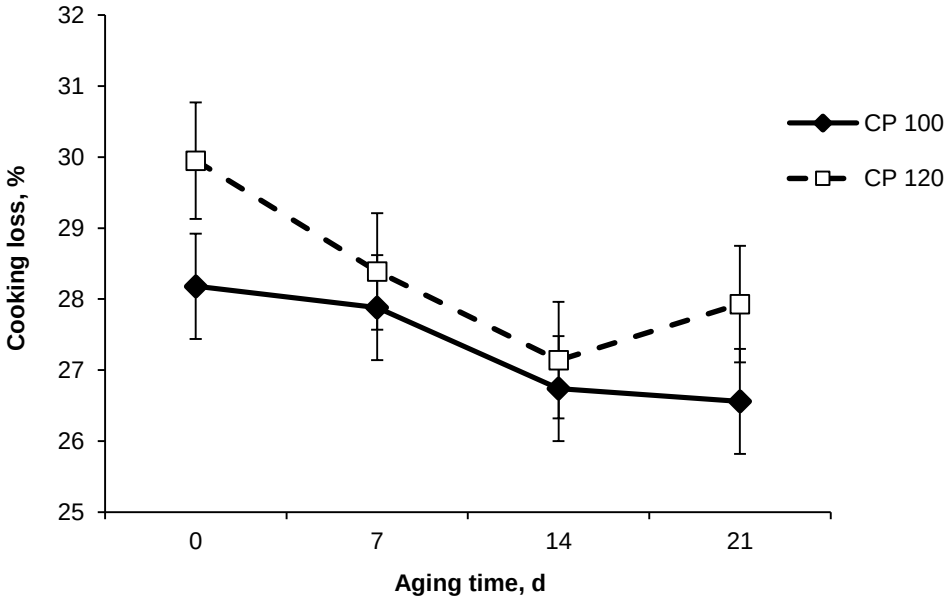
Table 5 - Effects of RH[1] and dietary protein content on carcass composition

Traits	CP 100[2]		CP 120[3]		SEM	*P*-Value		
	RH0	RH300	RH0	RH300		RH	CP	RHxCP[4]
Moisture, %	58.3	57.3	58.7	58.0	0.7	0.25	0.46	0.90
Protein, %	17.53	17.67	17.24	17.44	0.22	0.25	0.46	0.90
EE[4], %	19.5	20.7	18.9	19.9	0.9	0.25	0.46	0.90
Ash, %	4.35	4.28	4.38	4.33	0.05	0.25	0.46	0.90
Protein/EE	0.90	0.84	0.93	0.88	0.05	0.28	0.44	0.90

[1]RH = ractopamine hydrochloride (Ouro Fino Saúde Animal, Cravinhos, SP, Brazil) fed at 0 or 300 $mg \cdot animal^{-1} \cdot d^{-1}$ during 35 d before slaughter.
[2]Diet formulated to meet 100% of metabolizable protein requirements.
[3]Diet formulated to meet 120% of metabolizable protein requirements.
[4]EE: ether extract
Fonte: Cônsolo, 2015

Regarding meat quality, there was no effect of RH x CP interaction (*P*=0.26), or of RH supplementation (*P*=0.66)on cooking loss. There was a tendency (*P*=0.07) for greater cooking loss at CP120 than at CP100 (28.4 vs. 27.3% for CP120 and CP100, respectively, Figure 1), and this effect was not dependent on the postmortem aging time (*P*=0.77 for CP x Time interaction). Postmortem aging time altered cooking loss (*P*=0.04), as cooking loss decreased with aging time. After 14 and 21 days of aging, cooking losses were lower than at day 0 (*P*<0.05, Figure 1).

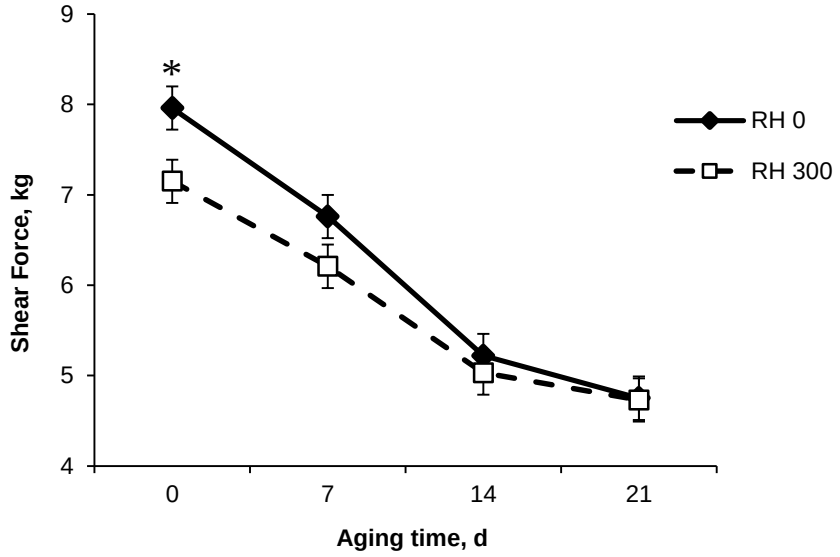
Figure 1 - Effect of dietary protein content and aging on cooking loss.



Fonte: Cônsolo, 2015

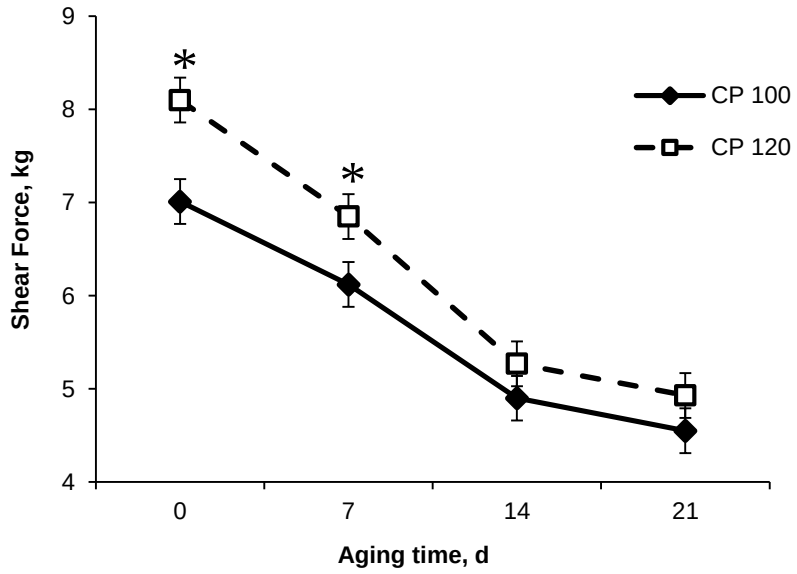
There was no RH x CP interaction for meat WBSF (*P*=0.13). There was a significant RH x Time interaction for meat WBSF (*P*<0.01, Figure 2). Decomposition of the Time x RH interaction demonstrated that RH supplementation decreased meat WBSF only at day 0 of aging (*P*=0.03), having no effect on WBSF after 7, 14 or 21 d of aging. There was also a CP x Time interaction for meat WBSF (*P*<0.01), with CP120 increasing WBSF after 0 and 7 d of aging (*P*<0.05), with no effect of dietary CP on meat WBSF after 14 or 21 d of aging (Figure 3).

Figure 2 - Effects of ractopamine hydrochloride (RH) and aging time on meat shear force.



Fonte: Cônsolo, 2015

Figure 3 - Effects of dietary protein content and aging time on meat shear force.



Fonte: Cônsolo, 2015

2.5 DISCUSSION

In feedlot systems, DMI, ADG, and G:F are crucial cost-effective traits that affect the time that animals spend on feed and total profitability. Previous studies have clearly

demonstrated the efficacy of adding β-AA to diets thereby improving cattle performance (VESTERGAARD et al., 1994; DUNSHEA et al., 2005; AVEDAÑO et al., 2006). Schroeder (2004) summarized results from 10 experiments completed in different regions of the United States, using RH at the same dietary level as in the current study during the finishing phase, and found that ADG, total BW gain, and the efficiency of gain were improved significantly by 26, 20, and 20.5 %, respectively, compared with non-supplemented controls. Nevertheless, these results were mostly conducted with *Bos taurus* breeds, with a high percentage of fat in the carcass during the RH supplementation period. There are very few data with the effect of RH supplementation in *Bos indicus* breeds with lower percentage of fat in the carcass.

In the present study with Nellore intact males, RH supplementation was also effective in increasing ADG and G:F ratio, but there was no effect on final BW. Avedaño et al. (2006) reported an increase of 24% in ADG, and 34% in the G:F in crossbred steers consuming 300 mg/d of RH. More recently, Bryant et al. (2012) found no difference in final BW of RH supplemented steer, but addition of RH improved ADG by 25%, and G:F ratio in steers consuming 200 mg/d of RH. Similarly, Schroeder (2004) has shown 12.4 to 23.4 % improvements in feed efficiency in steers fed 200 mg/d of RH for the final 28 d of the finishing period.

Because of the greater protein synthesis in ractopamine fed animals, a concomitant increase in protein requirement could be expected. According to the NRC (2000), increasing ADG from 1.4 to 1.6 kg/d results in approximately 12% increase in the metabolizable protein requirement for growth; therefore, animal response to ractopamine could benefit from extra dietary protein. Contrary to our hypothesis, increasing dietary CP above standard requirement had no effect on body gain or feed efficiency in the present study. Our results corroborate with that of Walker et al. (2006),

who suggested that feeding *β*-agonists could enhance the efficiency with which cattle use metabolizable protein, thereby leading to no change in protein requirements.

Ractopamine supplementation has been shown to decrease feed intake (AVENDAÑO et al., 2006; MCEVERS et al., 2012). In the present study, RH also reduced feed intake, but only when associated with a diet with excess CP. Because RH supplementation can reduce protein degradation, the increased amino acid (**AA**) supply to the liver could explain the reduction in feed intake. The greater oxidation of AA in the liver could generate excess ATP leading to reduced intake (ALLEN et al., 2009).

The effects of RH supplementation on blood metabolites were also dependent of dietary CP levels. Glucose plasma concentration can be reduced when there is an increase in production demand, such as the increase is muscle synthesis in RH supplemented animals. The fact that RH reduced plasma glucose only at the CP100 diet probably reflects the excess of gluconeogenic AA available at the liver in the CP120 diet. The increase in the efficiency of AA metabolism by RH supplementation can also explain the increase in plasma total protein associated with the CP120 diet. A lower muscle degradation rate, because of RH supplementation, can lead to reduced plasma urea concentration (WALKER et al., 2006; BRYANT et al., 2012), but this was not the case in the present study. Plasma activities of the liver enzymes AST, GGT and ALP are used as possible indicators of hepatic disturbances. Activities of AST, GGT, and ALP in the present study were within the range of normal values for cattle (RADOSTITIS et al., 2007), suggesting that RH supplementation had no negative effect on liver health status.

Increased muscle mass in mammals is recognized as an important effect of β-AA oral administration, by increasing the synthesis of muscle protein, reducing the

degradation of muscle protein, or a combination of both. This β-AA induced muscle hypertrophy is accredited to an increased rate of muscle α-actin synthesis, as well as to the inhibitory activity of calpastatin (SMITH et al., 1989; YANG and MCELLIGOTT, 1989). Fat deposition in several organs can also be usually affected by β-AA supplementation. Avedaño et al. (2006) reported 6% and 18% decrease in fat thickness of steers fed RH and ZH, respectively. In the present study, RH supplementation tended to increase LMA, but did not alter carcass fat thickness. Bryant et al. (2012) also reported no change in fat thickness when steers were supplemented with 200 mg/d of RH.

Contrary to the present study, carcass fat thickness is usually not affected by dietary protein concentrations, if there is no change in the total amount of energy in the diet (FLUHARTY et al., 2000). The main substrate for lipogenesis in cattle is acetate from rumen fermentation of carbohydrates; therefore, excess protein in the diet could be producing less acetate in the rumen and reducing fat synthesis in the adipose tissue. Associated with the reduced acetate, diets with excess protein can also decrease energy availability for fat synthesis due to the higher energy expenditure for urea synthesis in the liver (Agnew and Yan, 2000). The changes in fat thickness with excess protein in the diet were not enough to promote changes in total carcass chemical composition, as estimated by the 9–10–11th rib composition.

Because of the effects of β-AA supplementation on protein metabolism, such as reduced protein degradation and decreased proteolytic activity in the muscle tissue, in general, studies have shown a negative impact on meat tenderness (GEESINK et al., 1993; VESTERGAARD et al., 1994). Avedaño et al. (2006) reported an increase of 16% in WBSF in meat from steers fed zilpaterol hydrochloride, and 9% increase in meat WBSF from bulls fed RH. In the present study, there was no negative effect of

RH supplementation on meat tenderness, measured by WBSF. In fact, contrary to expected, RH supplementation reduced meat WBSF at the day of slaughter, with no effect after some postmortem aging time. Analysing WBSF of steaks after 14 d of aging, Arp et al. (2013) reported a dose response of RH, with no difference in WBSF values for meat from steers treated with 200 mg·animal^{-1}·d^{-1} compared to non-treated control. Steaks from steers fed zilpaterol hydrochloride or higher doses of RH (300 or 400 mg·animal^{-1}·d^{-1}) had greater WBSF values and were rated lower for overall tenderness than controls (ARP et al., 2013).

Miller et al. (2001) suggested a categorization of meat tenderness based on WBSF values, with intermediate meat having between 3.92 and 4.5 kg, and tough meat between 5.42 to 7.2 kg. According to this categorization, the meat from the bulls fed RH in the current study would still be classified as tough meat at day 0, likely reflecting the breed differences in meat tenderness. Brooks et al. (2009) reported that the longer the period of supplementation with ZH the lower the percentage of meat with WBSF values below 4.5 kg.

Meat tenderness at day 0, and after 7 d of postmortem aging, was also affected by dietary CP levels in the present study. Few studies have focused on the effects of dietary protein content on beef tenderness. Berge et al. (1993) observed reduced meat tenderness with increased amounts of muscle production, because of greater dietary protein content. Marino et al. (2011) also reported increased meat hardness with greater protein supplementation. Bulls fed RH had no change in meat colour; however, dietary CP promoted a strong increase in the b* variable, which measures the intensity of yellowness in the meat. According to Sirtori et al. (2014), changes on meat colour generated by altering the crude protein content of the diet might be partially owing to the fat content. This statement agrees with the report of Latorre et al. (2003), who

demonstrated that the increase in fat content was associated with a more intense colour of meat.

2.6 CONCLUSION

In summary, our results have demonstrated for the first time the efficacy of ractopamine supplementation to increase gain, improve feed efficiency, and increase loin muscle area in intact Nellore young bulls, with relatively low carcass fat content. These effects were not further improved by increasing dietary protein content above requirements, and there was no negative effect of ractopamine on meat tenderness.

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3 CHAPTER 2 / PAPER 2

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Zilpaterol hydrochloride improves feed efficiency and changes body composition in non-implanted Nellore heifers

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3.1 ABSTRACT

This research aimed to evaluate the effects of zilpaterol hydrochloride (**ZH**; MSD Animal Health, São Paulo, Brazil) on the performance, carcass traits, serum metabolites, body composition, and gain composition of non-implanted Nellore heifers. Nellore heifers (n = 72; average BW = 267 ± 16 kg; average 18 mo of age) were maintained in a feedlot system for 118 d. Heifers were separated into 2 groups: Control and Zilpaterol (**ZH**). The ZH group received zilpaterol hydrochloride (8.3 mg/kg diet DM) during 30 d with 3 d of withdrawal before slaughter. Heifers were allotted to 18 pens, nine pens per treatment, and assigned to a randomized block design. The animals were weighed, blood samples were collected, and subgroups of heifers were slaughtered at the beginning of supplementation and after 20 and 33 d to evaluate performance, blood metabolites, empty BW (**EBW**) and EBW composition. Hot carcass and kidney-pelvic fat weights were recorded at slaughter. At 24 h postmortem, carcasses were fabricated and the 9–10–11th rib section was removed from the primal rib to analyze moisture, protein, ash, and ether extract (**EE)** content in empty body (**EB)** and gain composition. Heifers fed ZH had gains in HCW that were 19.7 kg greater than controls, reflecting the 30% increase ($P < 0.01$) in ADG. There was no change in DMI, resulting in 20% greater G:F ratio ($P < 0.01$) for heifers fed ZH. Heifers supplemented with ZH had carcass dressing percentages that were 3% greater than controls ($P < 0.01$), and also there was a 19% reduction in kidney-pelvic fat ($P = 0.05$). Zilpaterol increased serum creatinine ($P < 0.01$), tended to increase ($P = 0.06$) serum triacylglycerol, decreased serum NEFA ($P = 0.04$), and tended to decrease ($P = 0.06$) serum glucose. The EBW composition was changed after 20 d of ZH supplementation ($P = 0.02$), with ZH increasing the moisture, ash, and protein contents, whereas carcass fat was decreased by ZH. Consequently, the carcass CP:EE ratio after 20 d

was increased (*P* = 0.03) by 24% with ZH supplementation. There was no change on EBW composition after 30 d of ZH supplementation (*P* = 0.17). Regarding carcass gain composition, ZH increased EBW gain (*P* = 0.02) by 842 g/d from d 0 to d 30, EB protein gain by 221 g/d (*P* = 0.05) from d 0 to d 20, and by 180 g/d (*P* = 0.01) from d 0 to d 33. In conclusion, ZH supplementation in non-implanted Nellore heifers altered the composition of body weight gain, promoting greater lean tissue deposition and improving feed efficiency.

Key words: beta-adrenergic agonist, *Bos indicus*, carcass composition, heifer, zilpaterol

3.2 INTRODUCTION

The efficient use of nutrients has become a crucial aspect of beef cattle production, which accentuates the importance of growth promoters, such as beta-adrenergic agonists (**β-AA**), in enhancing the efficiency of meat deposition. Finishing heifers in feedlots is a common practice that supplies the market system with meat of better quality (REDDY et al., 2015). Heifers deposit more fat in the carcass, compared with steers and bulls, and usually producing a more succulent and tender beef (REDDY et al., 2015). Heifers are also more docile animals than males, which also helps to improve beef quality by decreasing the risk of stress-related problems, such as contusions and DFD meat (SHACKELFORD et al., 1994). However, mainly because of lower muscle tissue deposition, non-implanted heifers have lower gain efficiency than males. Therefore, the use of growth promoters, such as zilpaterol hydrochloride (**ZH**), can improve feed efficiency, performance, and consequently profitability in feedlot heifers. In general, studies have documented the effects of ZH to promote skeletal muscle growth, to improve feed efficiency and performance, and to increase

dressing percentage (SCRAMLIN et al., 2010; ARP et al., 2014), therefore shifting composition of BW gain (DELMORE et al., 2010). However, most studies with ZH have focused on steers or bulls, with only a few studies reporting ZH effects on beef heifers (MONTGOMERY et al., 2009; ROBLES-ESTRADA et al., 2009).

There are several differences between *Bos indicus* and *Bos taurus* breeds. In general, *Bos taurus* breeds such as Angus and Hereford, reach puberty and the point of slaughter earlier than *Bos indicus* breeds, resulting in differences in growth composition and in carcass characteristics (RODRIGUES et al., 2002). The aim of this research was to evaluate the effect of feeding ZH to Nellore heifers on performance, carcass traits, serum metabolites, and body and gain composition.

3.3 MATERIALS AND METHODS

All animal procedures used in this study were conducted in accordance with the Institutional Animal Care and Use Committee Guidelines of the University of São Paulo, and approved by the FMVZ animal ethics committee (Protocol Number: 2026311013).

3.3.1 Experimental Site

The feedlot study was conducted at the Beef Cattle Research Laboratory, University of São Paulo, Pirassununga, located at the city of Pirassununga, state of São Paulo, in southeast Brazil from September 2012 to January 2013. The animals were in the feedlot for a total of 118 d, with the last 33 d as the experimental period.

3.3.2 Cattle

Seventy-two 18-mo old Nellore heifers with an average BW of 280 kg ± 22 were housed in concrete collective pens (4 animals/pen; 3.0 m wide × 9.0 m deep; 3.0 m of

linear bunk space) with ad libitum access to feed and water. Upon arrival, heifers were weighed, blocked into 3 groups according to initial BW, and randomly allocated to the pens (9 pens per treatment).

3.3.3 Management and Feeding

The heifers were fed twice daily, at 0800 h and 1400 h, a corn silage based diet, with a 35:65 forage:concentrate ratio (DM basis; Table 1). The diet was formulated to meet the requirements allowing for an ADG of 1.2 kg/d (NRC, 2000). Diets were offered for ad libitum intake; DMI was measured daily by weighing of offered feed and orts, and the amount offered was adjusted daily allowing for a minimum of 5% of orts during the experiment. Ingredients and orts were sampled weekly and pooled to determine chemical composition.

Table 1 - Composition and analyzed nutrient content (DM basis) of the finishing diet

Item	% of DM
Ingredient	
Corn silage	35.0
Ground corn	50.4
Soybean meal	5.0
Wheat meal	6.0
Trace mineral mixture[1]	3.6
Analyzed composition	
CP	15.5
NDF	28.0
TDN[2]	75.1
NEg[2], Mcal/kg DM	1.16

[1]The trace mineral mixture contained (per kg): Zinc 728 mg, Iron 221 mg, Fluorine (max.) 106 mg, Calcium 116 g, Selenium 3 mg, Phosphorus 14 g, Manganese 226 mg, Copper 221 mg, Cobalt 29 mg, Iodine 21 mg, Sodium 44 g, Sulfur 43 g, Potassium 47 g, CP (min.) 109%, NPN (equivalent protein, max.) 109%, Monensin Sodium 1,000 mg/kg.
[2]Estimated according to National Research Council (NRC, 2000).
Fonte: Cônsolo, 2015

3.4.4 Treatments and Weighing

All heifers received the same diet for 64 d, after which the animals started to receive 2 treatments: 1) Inclusion of 8.3 mg of zilpaterol hydrochloride per kg of diet DM (**ZH**; MSD Animal Health, São Paulo, SP, Brazil); and 2) Control without ZH

inclusion (**CT**). The ZH was included in the mineral premix and mixed with the concentrate before preparation of the total mixed ration. The dosage of ZH was calculated to provide around 0.165 mg of ZH/kg of BW. Supplementation with ZH lasted for 30 d, with 3 d of ZH withdrawal before slaughter. Heifers were weighed, after 16 h of feed restriction, upon arrival, at the beginning of ZH supplementation, after 20 d of ZH supplementation, and after 30 d of ZH supplementation.

3.4.5 Feed Samples

Feed samples were collected weekly during the morning feeding and frozen for subsequent analysis of DM and CP according to AOAC (2000) methods 930.15 and 988.05, respectively. Neutral detergent fiber was determined as in Van Soest et al. (1991) using 8 M urea and heat stable α-amylase (Sigma Chemical Co., St. Louis, MO), in an ANKOM A200 Fiber Analyzer (ANKOM Technology Corp., Fairpoint, NY). Acid detergent lignin and ADF were analyzed according to Van Soest and Robertson (1985).

3.4.6 Blood Sample Collection and Analysis

Blood was collected at the beginning and at the end of ZH supplementation by puncture of the coccygeal vein or artery, prior the morning feeding. Blood samples (10 mL) were collected into 10-mL tubes (BD Vacutainer, São Paulo, SP, Brazil), without anticoagulant, for the measurement of serum glucose, total protein, albumin, serum urea nitrogen, triacylglycerols, cholesterol, and NEFA. Serum parameters were analyzed colorimetrically according to standard procedures using commercially available diagnostic kits (Randox Laboratories, São Paulo, SP, Brazil) in an ABS-200 Automatic Biochemistry Analyzer (CELM, São Caetano do Sul, SP, Brazil).

3.4.7 Slaughter and Carcass Samples

Subgroups of heifers were slaughtered at different time points to evaluate heifer growth, empty BW (**EBW**) composition, and ZH effects on EBW composition. Upon arrival (d -64) and at the beginning of ZH supplementation (d 0), 2 heifers were randomly selected from each BW block (total of 6 heifers) for slaughter. Similarly, 12 heifers (4 heifers from each BW block, corresponding a total of 6 heifers from each treatment) were slaughtered after 20 d of ZH supplementation (d 20), with no ZH withdrawal. At the end of the experiment (d 33), all remaining 48 heifers were slaughtered, with 20 heifers (8 heifers from block medium initial BW, 6 from block low initial BW, and 6 from block high initial BW, corresponding to a total of 10 heifers per treatment) being used for determination of EBW composition.

Heifers were slaughtered after an 18-h fast at the University of São Paulo, according to normal commercial practice. Hot carcass and kidney-pelvic fat weights were recorded at slaughter. At 24 h postmortem, carcasses were fabricated and the 9–10–11th rib section was removed from the primal rib.

3.4.8 9–10–11th Rib Composition

The procedure developed by Hankins and Howe (1946) was used to mark the 9–10–11th rib section (**HH** section) on the left side of each carcass. The samples were frozen, cut with chainsaw and crushed in a large grinder (Frigmann Herman, Itupeva, SP, Brazil). Ground samples were freeze-dried to constant weight with a lyophilizer (Itasul Import and Instrumental Technical Ltda, Porto Alegre, RS, Brazil), thus obtaining the water content of the HH samples.

The water content of the HH section was used to estimate EBW composition using equations developed for Nellore cattle (PUTRINO et al., 2006). After calculation

of EBW composition, data was used to calculate composition (CP and EE) of EBW gain.

3.4.9 Statistical Analysis

All statistical analyses were conducted using SAS version 9.1.2 for Windows (SAS Inst. Inc., Cary, NC). Data were analyzed as a randomized block design using the MIXED procedure. The model included the fixed effect of treatment (0 or 8.3 mg ZH/kg diet DM), and the random effect of blocks. Pens were considered as the experimental units. Significance was declared at $P \leq 0.05$, and trends were considered at $P > 0.05$ and $P \leq 0.10$ for all analyses.

3.5 RESULTS

3.5.1 Performance and Carcass Traits

Initial BW was similar for the treatments, demonstrating homogeneity at the initial allocation (Table 2). Heifers supplemented with ZH had 11.8 kg greater BW gain ($P < 0.01$, Table 2) compared with control heifers. Supplementation with ZH increased total ADG, with a 20% increase in ADG between d 0 to d 20 ($P < 0.02$), and a 30% increase in ADG from d 0 to d 30 ($P < 0.01$, Table 2). Despite the increase in ADG, DMI was not affected by ZH supplementation ($P > 0.70$, Table 2). Therefore, the observed increase in ADG with ZH supplementation resulted in better efficiency of gain, with a 23% and 26% greater G:F ratio from d 0 to d 20 and d 0 to d 30, respectively ($P < 0.01$, Table 2).

Reflecting the greater BW gain, HCW gain of heifers supplemented with ZH was 19.7 kg greater ($P < 0.01$, Table 3) than control heifers at the end of the experiment. However, there was no effect of treatment ($P = 0.32$) on HCW at d 20 (Table 3). In addition, ZH supplementation increased carcass dressing percentage by 3.7% at d 20

(P = 0.02) and by 2.7% at d 33 (P < 0.01), and reduced kidney-pelvic fat (P = 0.05) by 19.3% at d 33, but ZH had no effect on kidney-pelvic fat at d20 (Table 3).

Table 2. Effects of Zilpaterol hydrochloride (ZH) on performance and intake

Trait	Treatment		SEM	*P*-value
	Control	ZH[1]		
BW, kg				
Initial	351	356	12.5	0.18
Final	387	404	17.0	0.01
BWgain	36.1	47.9	3.2	<0.01
ADG, kg/d				
d 0 to d 20	1.19	1.43	0.14	0.02
d 0 to d 30	1.13	1.47	0.10	<0.01
DMI, kg/d				
d 0 to d 20	8.28	8.19	0.45	0.73
d 0 to d 30	8.21	8.29	0.46	0.74
G:F				
d 0 to d 20	0.1420	0.1749	0.0091	<0.01
d 0 to d 30	0.1400	0.1764	0.0049	<0.01

[1] 8.3 mg ZH/kg DM (MSD Animal Health, São Paulo, São Paulo, Brazil).
Fonte: Cônsolo, 2015

Table 3 - Effects of Zilpaterol hydrochloride (ZH) on carcass traits.

Trait	Treatment		SEM	*P*-value
	Control	ZH[1]		
HCW, kg				
d 0[2]	197	197	5.2	--
d 20[3]	209	218	6.2	0.32
d 33[4]	212	232	5.5	0.02
HCW gain, kg				
d 0 to d 20	12.0	20.8	6.2	0.33
d 0 to d 33	14.9	34.6	5.5	0.02
Dressing, %				
d 0[2]	51.6	51.6	1.50	--
d 20[3]	53.6	55.6	0.49	0.02
d 33[4]	54.0	55.5	1.50	<0.01
Kidney-pelvic fat[5], kg				
d 0[2]	4.60	4.60	0.55	--
d 20[3]	5.48	4.22	0.53	0.12
d 33[4]	5.70	4.60	0.60	0.05

[1]8.3 mg ZH/kg DM (MSD Animal Health, São Paulo, São Paulo, Brazil).
[2]Animals slaughtered before ZH supplementation.
[3]Animals slaughtered after 20 d of ZH supplementation.
[4]Animals slaughtered after 30 d of ZH supplementation and 3 d of ZH withdrawal.
[5]Sum of perirenal, inguinal, and omental fat depots.
Fonte: Cônsolo, 2015

3.5.2 Serum Composition

Because of the changes observed in muscle and fat metabolism, changes were expected for serum metabolites with ZH supplementation. Regarding protein

metabolites, there was no effect (*P* > 0.39) of ZH on serum urea, total protein, or albumin concentration (Table 4). However, serum creatinine was increased (*P* < 0.01) with ZH supplementation (Table 4). Zilpaterol decreased NEFA (*P* = 0.04) and tended to decrease (*P* = 0.06) serum glucose concentration, whereas there was a tendency (*P* = 0.06) to increase serum triacylglycerol concentration (Table 4).

Table 4 - Effects of Zilpaterol hydrochloride (ZH) on serum metabolites

Metabolite	Treatment		SEM	*P*-value
	Control	ZH[1]		
Urea, mg/dL	36.3	35.0	0.99	0.39
Creatinine, mg/dL	1.65	1.85	0.043	<0.01
Total Protein, mg/dL	6.33	6.63	0.28	0.46
Albumin, mg/dL	3.1	3.13	0.25	0.93
NEFA, mmol/L	0.50	0.33	0.054	0.04
Triacylglycerol, mg/dL	25.1	30.6	1.9	0.06
Glucose, mg/dL	72.0	63.8	2.8	0.06
Cholesterol, mg/dL	194	208	8.2	0.27

[1]8.3 mg ZH/kg DM (MSD Animal Health, São Paulo, São Paulo, Brazil).
Fonte: Cônsolo, 2015

3.5.3 HH section Composition

The serial slaughter data demonstrated that there was an effect of treatment on EBW composition (Table 5). After 20 d of treatment, ZH supplementation increased (*P* = 0.02) carcass moisture, ash, and protein contents, whereas carcass fat was decreased by 14% with ZH supplementation (Table 5). Consequently, the carcass CP:EE ratio at d 20 was increased (*P* = 0.03) by 24% with ZH supplementation (Table 5). Despite the changes in EBW composition after 20 d of ZH supplementation, there was no effect of treatment on carcass chemical composition at the end of the period (*P* = 0.17, Table 5).

Table 5 - Effects of Zilpaterol hydrochloride (ZH) on carcass composition

Trait	Treatment		SEM	*P*-value
	Control	ZH[1]		
d 0[2], % of carcass				
Moisture	58.1	--	1.0	-
Protein	17.5	--	0.30	-
Ether extract	19.8	--	1.2	-
Ash	4.34	--	0.07	-
Protein/ether extract	0.90	--	0.08	-
d 20[3], % of carcass				
Moisture	55.3	57.8	0.65	0.02
Protein	14.2	15.3	0.3	0.02
Ether extract	23.0	20.0	0.79	0.02
Ash	3.54	3.82	0.07	0.02
Protein/ether extract	0.62	0.77	0.04	0.03
d 33[4], % of carcass				
Moisture	54.6	55.9	0.64	0.17
Protein	13.9	14.5	0.29	0.17
Ether extract	24.0	22.4	0.78	0.17
Ash	3.45	3.59	0.07	0.17
Protein/ether extract	0.58	0.66	0.04	0.19

[1]8.3 mg ZH/kg DM (MSD Animal Health, São Paulo, São Paulo, Brazil).
[2]Animals slaughtered before ZH supplementation.
[3]Animals slaughtered after 20 d of ZH supplementation.
[4]Animals slaughtered after 30 d of ZH supplementation and 3 d of ZH withdrawal.
Fonte: Cônsolo, 2015

3.5.4 Composition of gain

The EBW and empty body (**EB**) fat gain from d 0 to d 20 were not affected (*P* = 0.33) by ZH supplementation (Table 6). In contrast, the ZH supplementation increased (*P* = 0.05) EB protein gain by 221 g/d in the same period (Table 6). For d 0 to 33, ZH supplementation increased EBW gain by 842 g/d (*P* = 0.02) and EB protein gain by 180 g/d (*P* = 0.01, Table 6). However, no changes were observed on EB fat gain (*P* = 0.36, Table 6). These data show the efficiency of ZH on improving body protein gain and, consequently, on increasing lean tissue deposition.

Table 6 - Effects of Zilpaterol hydrochloride (ZH) on empty body gain composition

Trait	Treatment		Animals	SEM	*P*-value
	Control	ZH[1]			
EBW[2] gain, g/d					
d -64 to 0 [3]	935	--	6	290	--
d 0 to 20 [4]	1128	1743	12	430	0.33
d 0 to 33 [5]	822	1664	20	240	0.02
EBfat[2] gain, g/d					
d -64 to 0 [3]	360	--	6	147	--
d 0 to 20 [4]	734	400	12	17	0.20
d 0 to 33 [5]	573	679	20	82	0.36
EBprotein[2] gain, g/d					
d -64 to 0 [3]	120	--	6	51	--
d 0 to 20 [4]	70	291	12	71	0.05
d 0 to 33 [5]	42	222	20	44	0.01

[1]8.3 mg ZH/kg DM (MSD Animal Health, São Paulo, São Paulo, Brazil).
[2]EBW = empty BW; EBfat = empty body fat; EBprotein = empty body protein.
[3]Animals slaughtered before ZH supplementation.
[4]Animals slaughtered after 20 d of ZH supplementation, with no ZH withdrawal.
[5]Animals slaughtered after 30 d of ZH supplementation and 3 d of ZH withdrawal.
Fonte: Cônsolo, 2015

3.6 DISCUSSION

The results provide evidence that ZH improved growth performance, G:F, HCW, and dressing percent of non-implanted Nellore heifers. Most studies evaluating ZH effects on muscle growth have focused on steers (AVENDANO-REYES et al., 2006; VASCONCELOS et al., 2008; SCRAMLIN et al., 2010; ARP et al., 2014). Fewer studies were conducted with beef heifers (MONTGOMERY et al., 2009; ROBLES-ESTRADA et al., 2009; LEHESKA et al., 2009; RATHMANN et al., 2012), and very few studies conducted with *Bos indicus* breeds.

There are important differences in muscle and adipose tissue deposition between *Bos indicus* and *Bos taurus* British breeds, such as Angus, Hereford, and Shorthorn (CROUSE et al., 1989; RODRIGUES et al., 2002, HIGHFILL et al., 2012). In general, British breeds deposit more fat than *Bos indicus* breeds (CROUSE et al., 1989; RODRIGUES et al., 2002, HIGHFILL et al., 2012). Comparing *Bos taurus* (Hereford x Angus crosses) with *Bos indicus* x *Bos taurus* heifers of the same age and reared on the same feeding regimen, Highfill et al. (2012) reported that *Bos indicus* carcasses were lighter, had less fat cover, smaller ribeyes, and less intramuscular lipid.

Despite the differences in fat and muscle deposition, the present study reports that supplementing ZH to non-implanted Nellore heifers results in a response similar to that observed in *Bos taurus* steers and in *Bos taurus* implanted heifers.

The greater BW gain, ADG and HCW of heifers fed ZH likely reflects improved muscle tissue deposition. Therefore, the lower performance observed in non-implanted heifers, compared to bulls, steers or implanted heifers, can be counterbalanced by feeding ZH during 30 d before slaughter. Zilpaterol hydrochloride can increase muscle hypertrophy by reducing protein degradation (KOOHMARAIE et al., 2002). The decrease in protein degradation is likely modulated by elevation of calpastatin, suppressing m-calpain, resulting in greater muscle growth (KOOHMARAIE et al., 2002). Therefore, as demonstrated in this study, it is possible to use ZH to considerably increase the efficiency of meat production of non-implanted heifers.

Similar results were reported by Rathmann et al. (2012), who found an increase of 9.5% in ADG and 12.5% in G:F for heifers fed ZH (8.33 mg/kg DM) during 20 to 22 d before slaughter. Robles-Estrada et al. (2009) reported an increase of 59% in carcass-adjusted ADG, and a 57% increase in G:F for heifers fed ZH 30 d before slaughter. In addition, Montgomery et al. (2009) feeding ZH for 20 and 40 d, reported an 18% increase in ADG, and a 21% increase in G:F for heifers. The authors attributed these results to improved muscle mass deposition in ZH-supplemented heifers.

The improvement in carcass dressing percentage and the 19.3% reduction in kidney-pelvic fat in the present study corroborates the shift in nutrient metabolism from non-carcass components (kidney-pelvic fat, heart fat, organ weight, mesenteric fat, and hide weight) to carcass components in animals fed β-adrenergic agonists (ARP et al., 2014). Consequently, carcass and non-carcass measurements could explain the

increased dressing percentages reported following ZH use (MONTGOMERY et al., 2009; ROBLES-ESTRADA et al., 2009; RATHMANN et al., 2012; ARP et al., 2014).

Some studies reported no change in DMI when animals are fed with ZH (ELAM et al., 2009; ROBLES-ESTRADA et al., 2009; PARR et al., 2011), while others have reported a decrease in DMI after administration of ZH (REINHARDT et al., 2014; MONTGOMERY et al., 2009; MCEVERS et al., 2012). In the present study heifers fed ZH had similar DMI to control heifers, which, associated with the greater ADG, resulted in improved feed efficiency. The better G:F ratio of ZH-supplemented animals can be attributed to changes in the composition of body gain, which may be confirmed by changes in serum metabolite concentrations.

Because of the increase on muscle deposition in animals fed ZH, some changes on muscle metabolism are expected. Usually, studies have reported a decrease on serum urea by ZH supplementation (PARR et al., 2014; VAN BIBBER et al., 2015) which may reflect a decrease of protein catabolism in skeletal muscle (VAN BIBBER et al., 2015) or an increase in tissue N deposition (BRAKE et al. 2011). However, in the present study, creatinine was the single metabolite from protein metabolism changed by ZH supplementation. Serum creatinine concentration increased with ZH supplementation, likely reflecting the increased muscle mass of these animals. Creatinine is a nitrogenous compound produced from muscle creatine and phosphocreatine, molecules mainly contained in muscle, and is a marker for the total amount of muscle mass (RENNIE AND MILLWARD, 1983; VIRGILI et al., 1994).

Several studies have reported increased concentrations of serum NEFA in response to exposure to β-adrenergic agonists (EISEMANN et al., 1988; CHIKOU et al., 1991). Typically, increased concentration of serum NEFA indicates mobilization of fat stores (greater lipolysis) to provide energy support for the physiological functions of

other tissues (BRYANT et al., 2010). Contrary to expectations, in the present study the concentration of NEFA was lower in ZH-treated heifers than in the control group. These results suggest that muscle tissue could be using NEFA as an energy source, in response to the ZH-promoted greater muscle growth (DRACKLEY, 1999; KOONEN et al., 2005). Similar to the decrease in serum NEFA, serum glucose concentration was reduced by ZH treatment, which could also represent an increase in muscle energy demand for growth in ZH-supplemented animals.

Complementing the effect of ZH on lipid metabolism, serum triacylglycerol concentration was increased in animals supplemented with ZH. The transport of triacylglycerols to target tissues is made via chylomicrons that, when reaching the tissue, are broken down by the lipoprotein lipase enzyme into glycerol and fatty acids to be absorbed and stored by the cells (adipose tissue) or oxidized (muscles; LEHNINGER, 2008). The increase in serum triglycerides in ZH supplemented animals could indicate less activity of the lipoprotein lipase enzyme, thereby reducing lipogenesis and accumulation of adipose tissue, which could also explain the decrease on kidney-pelvic fat accumulation in the present study.

Body composition determination is important to complement information on nutritional modulation of growth. Several methods have been described for the estimation of body composition in cattle, and the chemical composition of the section between the 9-10-11 ribs can be used as a reliable estimator of empty body composition (HANKINS and HOWE, 1946; PUTRINO et al., 2006; GOULART et al., 2008).

Heifers on this study were slaughtered at d 0, d 20 and d 33 of ZH supplementation to evaluate the effects on EBW composition and on composition of tissue gain. The differences observed on EBW composition at d 20 reflects the change

in the composition of tissue gain. Heifers receiving ZH had greater EBW gain with greater protein gain and similar fat gain, generating a leaner carcass after 20 d of treatment.

At the end of the 30 d period, EBW composition was not different between treatments. Zilpaterol-treated heifers continued to deposit more protein than control animals throughout the experiment period. However, fat deposition was probably greater at the end of the 33-d period than from d 0 to d 20. The average CP:EE ratio in EBW gain from d 0 to d 20 was 0.72, whereas from d 0 to d 30 the CP:EE ratio in EBW gain was 0.33 (data from Table 6). The different CP:EE ration in EBW gain observed from d 0 to d 20, compared to d 0 to d 33, could explain the lack of difference in EBW composition after 33 d of treatment.

The greater protein deposition in ZH-treated heifers, especially from d 0 to d 20, promotes a more efficient BW gain during the feedlot period. Other authors have reported that 20 d of ZH supplementation would be sufficient to promote the desired changes in carcass and gain composition in heifers and steers (KELLERMEIER et al., 2009; RATHMANN et al., 2012).

3.7 CONCLUSION

In conclusion, ZH supplementation in non-implanted Nellore heifers altered the composition of BW gain, promoting greater lean tissue deposition and improving feed efficiency.

3.8 ACKNOWLEDGEMENTS

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4 CHAPTER 3 / PAPER 3

Meat production and palatability traits of Nellore heifers fed with zilpaterol hydrochloride

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4.1 ABSTRACT

This research aimed evaluate the effects of zilpaterol hydrochloride (ZH; MSD Animal Health) in carcass traits, subprimals yield, meat quality, and palatability traits on meat from Nellore heifers. The ZH supplementation did not change LMA, back fat, meat color, and cooking loss. There was a significant ZH x Aging interaction for meat Warner Bratzler Shear Force (WBSF). There were no differences between treatments for steaks aroma, beef flavor, and off-flavor. However, tenderness and juiciness were affected by ZH supplementation, depending on postmortem aging. Heifers fed ZH had an increase on hindquarter weight and carcass percent. Consequently, most muscles from hindquarter were heavier for animals fed ZH. Forequarter and brisket did not change with ZH supplementation. The ZH increased μ-calpain and m-calpain, and calpastatin genes expression of Nellore Heifers, with no changes for day of slaughtered and Day*Treatment interaction. In conclusion, ZH increases muscle growth and subpimals yield on Nellore heifers, and reduces meat quality under analyzed at 0, 7 or 14 days of postmortem aging with no effect after 21 days aging.

Keywords: beta-adrenergic agonist, meat quality, sensory panel.

4.2 INTRODUCTION

Growth promoters technologies, such as zilpaterol hydrochloride (ZH), is an alternative to improve feed efficiency, muscle growth and subprimal yield in beef cattle (SCRAMLIN et al., 2010; ARP et al., 2014). However, the beneficial effects on muscle mass deposition can lead to changes on meat quality and palatability traits. Meat palatability has been defined as tenderness, juiciness and flavor of cooked meat and is important to consumer satisfaction (MILLER, 2004).

Some authors have reported the ZH effect on performance and meat in *Bos taurus,* steers and bulls (QUINN et al., 2008; BECKETT et al., 2009; SCRAMLIN et al., 2010; ARP et al., 2014). However, few studies have reported the effect of ZH on meat palatability traits and subprimal yield in heifers (MONTGOMERY et al., 2009; LEHESKA et al., 2009; RATHMANN et al., 2012), and no study has reported ZH effect on *Bos indicus* cattle, such as Nellore.

Nellore cattle differ from Angus and Hereford breeds in important growth and meat quality parameters. Nellore reaches the point of slaughter later, with less carcass fat, and no marbling (VANCONCELOS et al., 2008; SCRAMLIN et al., 2010). The carcass differences alters the meat quality and palatability traits, especially tenderness and juiciness. Therefore, the aim of this study was to evaluate the effects of zilpaterol hydrochloride on carcass traits, subprimals yield, meat quality, and palatability traits on meat from Nellore heifers.

4.3 MATERIALS AND METHODS

All animal procedures used in this study were conducted in accordance with the Institutional Animal Care and Use Committee Guidelines of the University of São Paulo, and approved by the FMVZ animal ethics committee (Protocol Number: 2026311013).

4.3.1 Experimental Site

The feedlot study was conducted at the Beef Cattle Research Laboratory, University of São Paulo, located at the city of Pirassununga, state of São Paulo, in southeast Brazil from September 2012 to January 2013. The animals were in the feedlot for a total of 118 d, with the last 33 day of experimental period.

4.3.2 Cattle

Seventy-two 18 months old Nellore heifers with an average body weight (BW) of 280 kg ± 22 were housed in concrete collective pens (4 animals/pen; 3.0 m wide × 9.0 m deep; 3.0 m of linear bunk space) with *ad libitum* access to feed and water. Upon arrival, heifers were weighed, blocked in three groups according to initial weight, and randomly allocated to the collective pens (9 pens per treatment).

4.3.3 Management feeding and treatments

The heifers were fed twice daily, at 0800 h and 1400 h, a corn silage based diet, with a 35:65 forage:concentrate ratio (Table 1). The diet was formulated to meet the requirements allowing an average dairy gain (ADG) of 1.2 kg/day (NRC, 1996). The amount of feed offered was adjusted daily according to the weight of the orts, to allow a minimum of 3 % and a maximum of 5 % of orts.

Table 1 - Composition and analyzed nutrient content (DM basis) of the finishing heifers diet.

Item	% DM basis
Ingredient	
Corn Silage	35.0
Ground corn	50.4
Soybean meal	5.0
Wheat meal	6.0
Trace mineral mixture[1]	3.6
Analyzed composition	
CP	15.5
NDF	28,0
NDT[2]	75.1
NEg, Mcal/kg DM[2]	1.16

[1]The trace mineral mixture contained (per kg): Zinc 728mg, Iron 221mg, Crude Protein (min.) 109%, Fluorine (max.) 106mg, Calcium 116g, Selenio 3mg, Phosphorus 14g, Manganese 226mg, Copper 221mg, Cobalt 29mg, Iodine 21mg, Sodium 44g, Sulfur 43g, Potassium 47g, NNP - Equiv. Protein (max.) 109%, Monensin Sodium 1.000mg/kg.
[2]Estimated according to National Research Council (NRC, 2000).
Fonte: Cônsolo, 2015.

All heifers received the same diet for 85 days, after which the animals started to receive two treatments: ZH) Inclusion of 8.3 mg of zilpaterol hydrochloride (MSD Animal Health, Brazil) per kg of diet dry matter (DM); and CT) Control without ZH

inclusion. The ZH was included in the mineral premix and mixed with the concentrate before preparation of the total mixed ratio. Supplementation with ZH lasted for 30 days, with extra 3 days of ZH withdrawn before slaughter.

4.4.4 Slaughter and carcass samples

Subgroups of heifers were slaughtered at different time points to evaluate ZH effect on muscle back fat and Longissimus lumborum (LL) area. At the beginning of ZH supplementation (d 0), two heifers were randomly selected from each BW block (total of 6 heifers) for slaughter. Similarly, 12 heifers (6 from each treatment) were slaughtered after 20 days of ZH supplementation (d 20). At the end of the experiment (d 33), all heifers were slaughtered, with 20 heifers (10 per treatment) being used for determination of carcass composition.

Animals were slaughtered after 18 hours fasting at the University of São Paulo, experimental abattoir according to normal commercial practice. At slaughter, the hot carcass weight (HCW) was measure, and LL samples were taken to mRNA expression analysis. After 24 h postmortem, LL area, LL color, and back fat were recorded. In addition, at the last slaughter, the subprimal cuts were weighed to measure the subprimal yield. In addition, steaks were sample (2.5 cm thick) from LL of each animal, vacuum packed and aging for 0, 7, 14 or 21 day, and then frozen at -18 °C for posterior analysis of sensory panel, cooking loss (CL) and Warner Bratzler Shear Force (WBSF).

4.4.5 Meat quality analysis

The LL color was measure at the level of the 12th thoracic vertebra using a Minolta CR 200b in the L*, a* and b* system. Meat quality measure of WBSF and CL were determined at the Laboratory the Animal Evaluation and Meat Quality of the

University of São Paulo, using the methodology proposed by Wheeler et al. (2005) and previously described by Cônsolo et al. (2015). The steaks were thawed for 24 h at 4 °C, weighed, and roasted in an oven equipped with a thermostat adjusted to 170 °C (Flexa de Ouro Industry, São Paulo, SP, Brazil). The steaks internal temperature was monitored using individual thermometers (Globo Industry, Americana, SP, Brazil) until it reached 71 °C. The steaks were cooled to 28 °C and weighed again, thus obtaining the value for CL. Steaks were cooled at 4 °C for 24 h before shearing. For WBSF evaluation, six cores with 1.3 cm of diameter were taken from each steak, parallel to the orientation of the muscle fibres (Ferrari furadeira, São Paulo, SP, Brazil). Each core was sheared perpendicular to the muscle fibre using a WBSF instrument (Warner-Bratzler meat Shear, G-R Manufacturing, Collins, KS, USA), according to standard procedures from American Meat Science Association (AMSA, 1995). The WBSF values of the six subsamples were averaged for statistical analysis.

4.4.6 Sensory Analysis

The sensory analysis was performed at the Laboratory of Sensory Analysis of the University of São Paulo. Beef sensory characteristics were assessed by a panel of 10 trained members (AMSA, 1995), previously described by Cônsolo et al. (2015). Five sessions were performed, including three training sessions, one blank test, and analysis test. Each member evaluated two samples per treatment (CT and ZH) and per postmortem aging time.

Steaks (approximately 2.5 cm thick) were thawed at 4 °C and cooked as described for WBSF and CL analysis . The oven was pre-heated with a thermostat adjusted to 170 ºC and the internal temperature of the steaks was monitored individually. Each steak placed on a metal rack over an aluminum tray was turnover

after reached an internal temperature of 40 ºC. Steaks were removed from the oven when they had reaching an internal temperature of 71 ºC. Grilled steaks were cut immediately into 1 cm cubes which were transferred to glass flasks with metal lids. A yogurt maker equipped with a thermostat adjusted to 40 °C was used to keep samples warm until analysis. The samples were analyzed in individual booths under controlled conditions of light and temperature (MEILGAARD et al., 1999).

The ten-member trained sensory panel evaluated the tenderness, juiciness, intensity of beef aroma, beef flavor and beef off-flavor of the samples on an 8 point scale, where 8 = extremely tender, juicy and intense, and 1 = extremely tough, dry and absent (AMSA, 1995).

4.4.7 Isolation of RNA and Quantitative PCR

Longissimus lumborum samples were taken immediately after animal slaughtered, and frozen in liquid nitrogen. The total RNA extraction was performed using Trizol reagent (Invitrogen - Life Technologies, São Paulo, SP, Brazil) with adaptation of the method described by Chomczynski and Sacchi (1987). A NanoDrop was used to spectrophotometrically quantify RNA, and RNA integrity was validated by the OD260/OD280 absorption ratio (>1.8) as previously described by Korna et al. (2013). Total RNA (2µg/reaction) was incubated with DNase I according to the manufacturer's protocol. Complementary DNA was synthesized using a Superscript III according to manufacturer´s instructions (Invitrogen- Life Technologies, São Paulo, SP, Brazil).

Individual real-time PCR reactions were performed in 96-well plates on 7500 Real Time PCR System containing 10 µL of 2x SYBR Green Master Mix (Life Technologies, Foster City, CA), 0.30 µM concentration of each primer (Table 2), and 1 µL of cDNA

template in a final volume of 20 µL per reaction. The samples were assayed in duplicate . Genes evaluated included µ- and m-calpain (CAP1 and CAP2, respectively), and calpastatin (CAST). In addition, the beta-actin and 18S genes were used as endogenous. Primers were ordered from Integrated DNA Technologies, Inc.Primer (IDT). Primer sequences and accession numbers are listed in Table 2. The PCR amplification protocol was as follows: an initial denaturation step at 95°C for 10 min, then 44 cycles of heating and cooling at 95°C for 15 s and 60°C for 30 s, and extension at 72°C.Melting curves were analyzed at the end of the reactions to verify the specificity of each amplification.

Table 2. Sequences and accession numbers of PCR primers

Gene	Sequence 5' - 3'
18SrRNA (NM_001001443)	
Forward	CGGCGACGACCCATTCGAAC
Reverse	GAATCGAACCCTGATTCCCCGTC
β-actin (BT030480)	
Forward	CATCCGCAAGGACCTCTAC
Reverse	ATGCCAATCTCATCTCGTTTT
Calpastatin (AF159246.1)	
Forward	CCCTGGATCAACTTTCTGACAGT
Reverse	TGACTTTATCCTCTACAGGTTTATTCTCA
µ-calpain (BC123635.1)	
Forward	GAATTACCTGTCCATCTTCC
Reverse	GATCAGTGTCCAGAGTTTTG
m-calpain (NM_001103086.1)	
Forward	ATGCAGCTATGACATCTACC
Reverse	TCCAGCACTTGAGTTAAGAC

Fonte: Cônsolo, 2015.

4.4.8 Statistical Analysis

All statistical analyses were conducted using SAS version 9.1.2 for Windows (SAS Institute Inc., Cary, NC). Data were analyzed as a completely randomized block design using the MIXED procedure. The model included the fixed effect of treatment

(0 or 8.3 mg ZH/animal/day), and the random effect of blocks. Animals were considered as the experimental units and included in the model as a random effect. Treatment means were compared using Fisher's least significant difference (PDIFF in the LSMEANS command). Sensorial data was considered nonparametric and was performed by the Kruskal– Wallis test using the PROC NPAR1WAY. In all comparisons, significance was declared at $P \leq 0.05$.

4.5 RESULTS

4.5.1 Meat quality

Supplementation with ZH did not change LL area ($P = 0.10$) and back fat ($P = 0.54$, Table 3). Meat color was unaffected ($P > 0.05$) by ZH supplementation (Table 3). According to Muchenje et al. (2009), the L*, a* and b* values for beef range from 33.2 to 41.0, from 11.1 to 23.6, and from 6.1 to 11.3, respectively. Thus, the values observed in the present study were within the normal range.

Table 3 - Effects of zilpaterol hydrochloride[1] on meat quality.

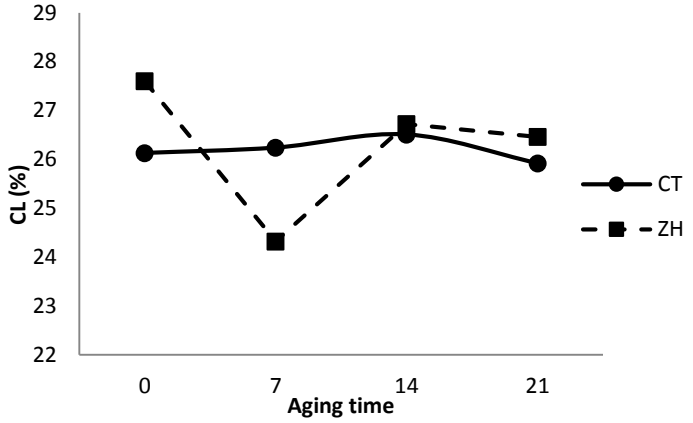
Trait	Diets[2]		SEM	*P*-Value
	CT	ZH		
LL area, cm^2	73.10	83.50	4.20	0.100
BF, mm	5.20	4.80	0.48	0.540
Color[3]				
L*	34.70	33.71	0.67	0.479
a*	18.49	19.02	0.64	0.590
b*	10.85	11.07	0.32	0.744

[1]Zilpaterol hydrochloride (MSD Animal Health, São Paulo, São Paulo, Brazil).
[2]Diets: CT = control (with 0 mg ZH/kg DM of ZH); ZH = with 8.3 mg ZH/kg DM.
[3]L* = Lightness; a* = redness; b*=yellowness. Reference values between 33.2 to 41.0; 11.1 to 23.6 and 6.1 to 11.3 for L*, a* e b*, respectively, according to Muchenje et al. (2009).
Fonte: Cônsolo, 2015.

Zilpaterol hydrochloride and postmortem aging did not change cooking loss ($P > 0.05$); however, there was a significant ZH x Aging interaction for meat WBSF ($P =$

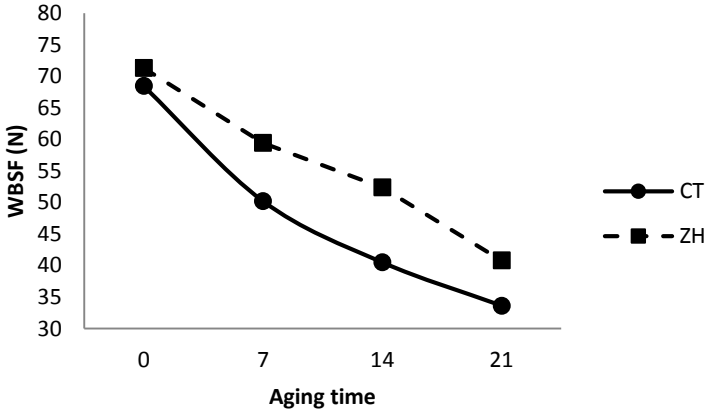
0.03, Figure 1). Decomposition of the ZH x Aging interaction demonstrated that ZH supplementation increased by 18 % and by 29 % meat WBSF at 7 and 14 days postmortem aging, respectively, with no effect on WBSF at 0 or 21 days of postmortem aging (Figure 2). This lack of difference at 21 days of aging, indicates that the tenderization process was effective for both treatments.

Figure 1 - Effects of zilpaterol hydrochloride (ZH) and postmortem aging on meat cooking loss (CL).



Fonte: Cônsolo, 2015.

Figure 2 - Effects of zilpaterol hydrochloride (ZH) and postmortem aging time on meat Warner Bratzler Shear Force (WBSF).



Fonte: Cônsolo, 2015.

4.5.2 Subprimal yield

Heifers fed ZH had a 13 % increase on hindquarter weight (*P* < 0.01, Table 4), with no difference for forequarter (P = 0.26; five ribs) and brisket weight (P = 0.66) between treatments. Consequently, most muscles from hindquarter were heavier for animals fed ZH, including top sirloin cap (*P* = 0.01), striploin (*P* = 0.05), tenderloin (*P* < 0.01), eye of rump (*P* < 0.01), knuckle (*P* < 0.01), inside round (*P* < 0.01), outside round (*P* = 0.01) and eye of round (*P* = 0.04).

Table 4 - Effects of zilpaterol hydrochloride[1] on subprimal yield.

Trait	Diets[2]		SEM	Valor-P
	CT	ZH		
	kg of carcass			
Hindquarter	48.4	54.8	2.7	<0.01
Forequarter (5 ribs)	40.1	41.5	2.0	0.26
Brisket	17.2	17.5	1.3	0.66
Top sirloin cap	1.53	1.75	0.07	0.01
Striploin	6.2	6.9	0.4	0.05
Tenderloin	1.78	2.15	0.15	<0.01
Eye of rump	4.00	4.68	0.23	<0.01
Knuckle	4.21	4.95	0.19	<0.01
Inside round	7.4	9.0	0.3	<0.01
Outside round	4.04	4.97	0.21	0.01
Eye of round	1.92	2.21	0.10	0.04
	% of carcass			
Hindquarter	45.60	48.00	0.30	0.02
Forequarter (5 ribs)	37.80	36.20	0.27	0.05
Brisket	16.14	15.20	0.27	0.06
Top sirloin cap	1.46	1.52	0.02	0.24
Striploin	5.80	6.00	0.08	0.58
Tenderloin	1.66	1.88	0.03	<0.01
Eye of rump	3.76	4.06	0.04	0.01
Knuckle	3.98	4.32	0.02	<0.01
Inside round	6.98	7.84	0.06	<0.01
Outside round	3.82	4.34	0.05	<0.01
Eye of round	1.80	1.92	0.03	0.20
Debone yield	72.20[a]	73.6[b]	0.50	<0.01

[1]Zilpaterol hydrochloride (MSD Animal Health, São Paulo, São Paulo, Brazil).
[2]Diets: CT = control (with 0 mg ZH/kg DM of ZH); ZH = with 8.3 mg ZH/kg DM.
Fonte: Cônsolo, 2015.

Similarly, when the subprimals were expressed as percentage of carcass (Table 4), ZH supplementation increased the percentage of hindquarter in the carcass by 2.4 percentage units (*P* = 0.02), decreased forequarter by 1.6 percentage units (*P* = 0.05; five ribs), and no effect (*P* = 0.06) to brisket percentage. Within hindquarter subprimals, ZH increased tenderloin (*P* < 0.01), eye of rump (*P* = 0.01), knuckle (*P* < 0.01), inside

round (*P* < 0.01), outside round (*P* < 0.01) percentage in the carcass. In addition, ZH improved by 1.9 percentage units the debone yield (*P* < 0.01; Table 4).

4.5.3 Palatability traits

There were no differences between treatments for steaks aroma, beef flavor, and off-flavor (*P* > 0.05, Table 5) regardless of postmortem aging. However, the panelist were capable to differ meat tenderness from heifers fed CT and ZH (*P* < 0.05, Table 4) in all aging time, where ZH decreased tenderness score by 37 % on compared with CT.

Table 5 - Effects of zilpaterol hydrochloride[1] and postmortem aging on meat sensory test.

Trait	Diets[2]		SEM	*P*-Value
	CT	ZH		
Aging 0 d				
Texture	5.8	2.88	0.34	<0.01
Juiciness	6.11	5.05	0.20	0.01
Aroma	5.77	5.88	0.10	0.67
Beef flavor	5.77	5.94	0.08	0.36
Off-Flavor	1.55	1.38	0.12	0.60
Aging 7 d				
Texture	5.38	3.35	0.25	<0.01
Juiciness	5.22	4.61	0.19	0.13
Aroma	5.88	5.88	0.07	1.00
Beef flavor	5.94	5.77	0.10	0.41
Off-Flavor	1.55	1.5	0.10	0.79
Aging 14 d				
Texture	6.27	4.37	0.27	<0.01
Juiciness	5.44	4.12	0.26	0.01
Aroma	6.22	5.87	0.08	0.09
Beef flavor	5.88	5.87	0.14	0.96
Off-Flavor	1.72	1.5	0.14	0.53
Aging 21 d				
Texture	6.83	4.77	0.27	<0.01
Juiciness	5.55	4.88	0.20	0.19
Aroma	6.05	6	0.10	0.83
Beef flavor	5.72	6	0.07	0.16
Off-Flavor	2.11	1.5	0.15	0.14

[1]Zilpaterol hydrochloride (MSD Animal Health, São Paulo, São Paulo, Brazil).
[2]Diets: CT = control (with 0 mg ZH/kg DM of ZH); ZH = with 8.3 mg ZH/kg DM.
Fonte: Cônsolo, 2015.

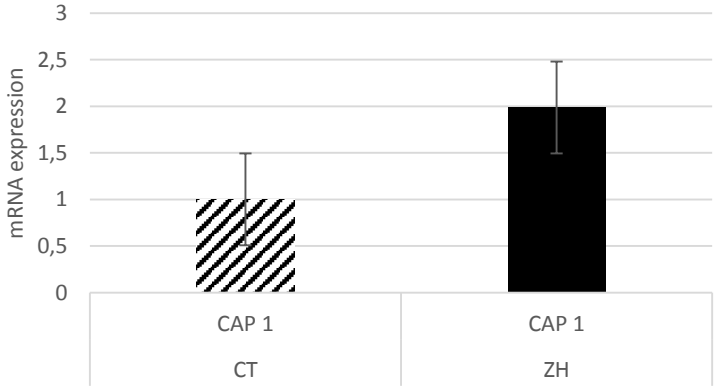
In addition, the postmortem aging from 0 to 21 day increased tenderness (*P* < 0.05) by 18 % and by 65 % for CT and ZH treatments, demonstrating the efficiency of postmortem aging to increase tenderness regardless of treatment. Regarding

juiciness, steaks from ZH treated heifers received scores that were 17 % and 24 % lower at 0 and 14 days aging, respectively, compared, with steaks from CT heifers (*P* < 0.05, Table 5). No differences were reported at 7 and 21 days aging between treatments.

4.5.4 Gene expression

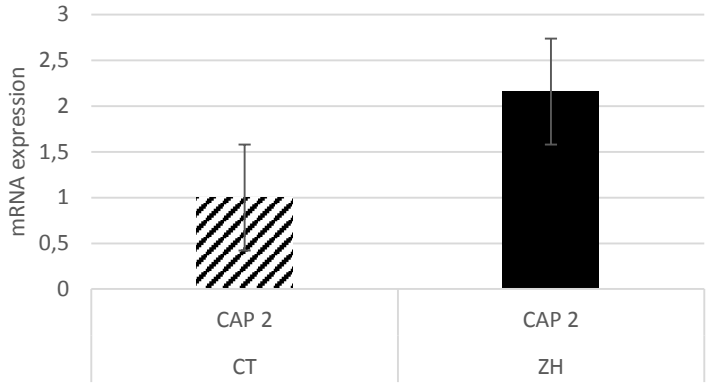
There were no effect of day of slaughtered (Day) and Day*Treatment interaction for genes expression (*P* > 0.05). However, heifers fed ZH changed the calpain/calpastatin system expression. The ZH supplementation increased µ-calpain by 7.8 % (*P* = 0.036), m-calpain by 9.0 % (*P* = 0.016), and tended to increase 8,81 % (*P* = 0.08) on calpastatin (Figure 3, 4 and 5 respectively).

Figure 3 - Effects of zilpaterol hydrochloride (ZH) on µ-calpain RNA expression.



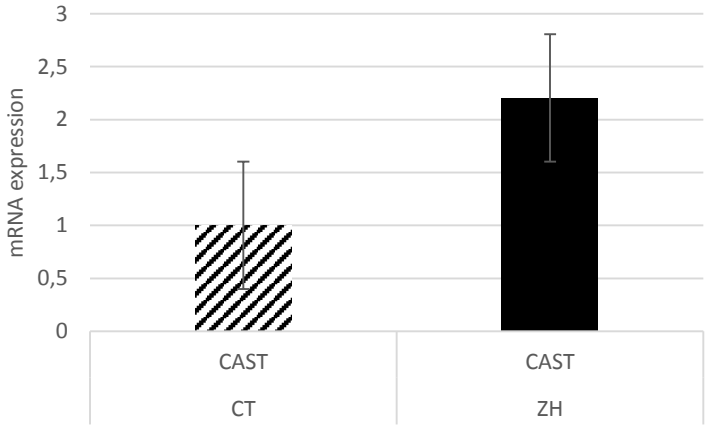
Fonte: Cônsolo, 2015.

Figure 4 - Effects of zilpaterol hydrochloride (ZH) on m-calpain RNA expression.



Fonte: Cônsolo, 2015.

Figure 5 - Effects of zilpaterol hydrochloride (ZH) on calpastatin RNA expression.



Fonte: Cônsolo, 2015.

4.6 DISCUSSION

Several studies have reported the effect of beta-adrenergic agonists (β-AA), such as ZH, on beef cattle performance (Holland et al., 2010; Parr et al., 2010; Arp et al., 2014); however, less emphasis has been given to the effect of β-AA on meat quality parameters including sensory attributes, subprimals yield and gene expression. Zilpaterol hydrochloride induces muscle hypertrophy and, consequently, meat toughness in Bos taurus breeds, (Brooks et al., 2009; Leheska et al., 2009; McEvers

et al., 2012). However, there is no data reporting the effect of ZH in meat quality, subprimal cuts yield and gene expression of Bos indicus breeds, such as Nellore.

The current study provides evidence that ZH influence the increase on muscle deposition and product yield of Nellore heifers. This result may be due to increased muscle protein synthesis, reduced degradation of muscle protein, or a combination of both (Yang and McElligott, 1989). Improved efficiency of protein deposition and of beef production are of great importance to all livestock industries (Kellermeier et al., 2009). The β-AA promoted increase on muscle deposition, which can be measure by subprimals yield. In this study, ZH supplementation increased hindquarter weight by 13 % and by 5 % on carcass percentage. Consequently, ZH increased almost all subprimals cut yields from hindquarter, regardless measuring it as kg or as % of carcass. In addition, ZH increased the debone yield, demonstrating the efficiency of ZH to improve meat production. Moloney et al. (1990) reported that L644,969 increased the cutability of low and high priced cuts including the brisket, strip loin, inside, and outside round. In general, our results are in agreement with Hilton et al. (2009) and Kellermeier et al. (2009) in that carcass cutout yield was most pronounced in the hindquarter of ZH supplemented cattle. This results show the efficiency of ZH increases protein deposition mainly in hindquarter subprimals cut. In summary, Nellore heifers in feedlot present a great performance and muscle growth when fed with ZH, provide evidences that ZH increase beef productivity as consequently improve meat industry profitability.

Consumers have identified tenderness as the most important meat palatability trait (Platter et al., 2003). They can discern differences between categories of tenderness based on WBSF and are willing to pay more for improved tenderness (Boleman et al., 1997; O´Quinn et al., 2012). This study demonstrated that ZH

supplementation increased meat WBSF by 9.21 and by 11.86 N at 7 and 14 days postmortem aging, compared with CT. It has been suggested that, ZH supplementation increases WBSF due to an increase in the calpastatin activity and a reduction in both μ-calpain and m-calpain activity (Koohmaraie et al., 2002). Nevertheless, differences between treatments can be minimize by postmortem aging. The present study reports a decrease in WBSF as days postmortem increases regardless treatment. Consequently, after 21 days postmortem aging, WBSF of ZH treated heifers were down to similar levels as control heifers.

Eating satisfaction results from the interaction of tenderness, juiciness, aroma, and flavor. Therefore, tenderness variation may be the most important attribute that can change meat quality. In the present study, a panel compounded by 10 trained members were able to identify difference in meat tenderness between treatments at all postmortem aging. Zilpaterol hydrochloride decreased by 36 % LL tenderness compared with CT treatment (6.07 and 3.84 panellist score average for CT and ZH respectively). The lower tenderness for animals fed ZH can be due to the ZH effect in increasing muscle hypertrophy and reducing protein degradation, as previously reported (Koohmaraie et al., 2002).

Panellist tenderness scores were significantly improved with postmortem aging, regardless of treatment. However, in all aging times, tenderness scores were lower for ZH than for CT treatment. Some authors reported that postmortem aging greater than14 days decreases tenderness disparities between steaks from cattle fed beta-adrenergic agonists and CT (Scramlin et al., 2010; Boler et al., 2012). Nevertheless, the aging period required to eliminate the effect of ZH on meat tenderness is still variable among studies (Hilton et al., 2009; Kellermeier et al., 2009).

Juiciness, as tenderness, is also an attribute valued by most consumers. The present study reports a ZH promoted decrease on juiciness score at 0 and 14 days postmortem aging compared with CT treatment. There contradictory results in the literature about the effects of ZH on juiciness, because this attribute depends mostly of aging time and meat fat content. Hilton et al. (2009) reported that trained panelists rated steaks from cattle fed ZH lower for tenderness and juiciness. Similary, Brooks et al. (2010) reported a decrease on tenderness scores, and a tendency to decrease juiciness when animals were fed with ZH compared to control. In summary, evidences indicates that ZH induces muscle growth by reducing protein degradation, consequently, decreases meat tenderness of treated animals. However, technologies, such as postmortem aging, can be used to eliminate the negative effect of ZH on meat quality, and improve meat tenderness.

The calpain/calpastatin system play a key role of meat tenderization, calpastatin inhibits both μ-calpain and m-calpain and requires calcium concentrations close to, or below, those required to activate calpain (KEMP et al., 2010). High calpastatin expression is correlated with muscle hypertrophy and meat toughness, and some authors have reported increases in calpastatin activity due to β-AA feeding (BARDSLEY et al., 1992; STRYDOM et al., 2009, 2011; HOPE-JONES et al., 2010). In the current study, both analysis to measure tenderness (WBSF and sensory test) have shown a decrease in beef tenderness by ZH supplementation, which could be explained by the increase on calpastatin mRNA expression, as consequence a decrease on protin degradation by calpain inhibition (BROOKS et al., 2009; LEHESKA et al., 2009; MCEVERS et al., 2012). According Koohmaraie et al. (2002) when muscle hypertrophy occurs by decrease on protein degradation (increases on calpastatin action), results an increased on meat WBSF, supporting our results.

The association between increased in calpastatin activity and WBSF values for animals fed with β-AA appears to be in agreement with reports of Geesink et al. (1993), Koohmaraie and Shackelford (1991), Wang and Beerman (1988), Simmons et al. (1997), Hope-Jones et al., (2010), and Strydom et al., (2009). However, reports on β-AA effects on calpain mRNA expression and activity are not consistent. In the present study, the calpain, as calpastatin, were increased by ZH supplementation; however, calpain could not improve the beef tenderness due to the excessive calpastatin activity, and consequently the calpain inhibition. Some author have reported, no changes com calpain mRNA expression or activity when animals are fed with β-AA (Strydom et al., 2009; Hope-Jones et al., 2010). But contrary to our results, Korn et al. (2013) demonstrates that μ-calpain mRNA expression tended to decrease due to ZH treatment. The authors explain that ZH may prevent an influx of ionized calcium into muscle, lend to a decrease in μ-calpain mRNA expression or activity, or potentially an increase in calpastatin activity as seen by others (Bardsley et al., 1992; Strydom et al., 2009, 2011; Hope-Jones et al., 2010).

4.7 CONCLUSION

In conclusion, ZH improve subprimal cut yield and debone yield, as consequence increase meat production in Nellore heifers. In addition, ZH decrease tenderness before 21 d aging and increase calpastatin, μ-calpain and m-calpain mRNA expression of Nellore heifers.

4.8 ACKNOWLEDGEMENTS

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5 GENERAL CONCLUSION

These results indicate the efficiency of RAC and ZH to improve the performance, feed efficiency, and muscle mass deposition in bulls and heifers Nellore. Greater CP did not maximize the RAC effect. The ZH supplementation increase the subprimal cut yields and change body gain composition. Finally, the use of β-adrenergic agonist increase the beef cattle system efficiency by increase the lean carcass component, with greater profitability per animal slaughtered.

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