



Zilpaterol hydrochloride improves beef yield, changes palatability traits, and increases calpain-calpastatin gene expression in Nellore heifers



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ABSTRACT

This research aimed to evaluate the effects of the beta-agonist zilpaterol hydrochloride (ZH) on carcass traits, subprimal yield, meat quality, palatability traits, and gene expression in Nellore heifers. Zilpaterol increased *Longissimus lumborum* area and did not change back fat thickness, meat color, and cooking loss. Heifers fed ZH had greater hindquarter weight and carcass percentage. Muscles from hindquarter were heavier for animals fed ZH. Forequarter (% of carcass) decreased and brisket did not change with ZH supplementation. There were no differences between treatments for steak aroma, beef flavor, and off-flavor. However, tenderness and juiciness were reduced by ZH, depending on postmortem aging. Zilpaterol increased Calpain-1, Calpain-2, and calpastatin mRNA expression, with no effect of day of slaughter or ZH × Day interaction. In conclusion, ZH supplementation improved hypertrophy, meat production, and debone yield in Nellore heifers, which led to decreased tenderness and to increased mRNA expression in the calpain-calpastatin system.

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1. Introduction

Growth promoter's technologies, such as zilpaterol hydrochloride (ZH), is one way to improve feed efficiency, muscle growth and subprimal yield in beef heifers (Scramlin et al., 2010; Arp et al., 2014). The skeletal muscle hypertrophy in animals fed with ZH is correlated with a decrease in protein degradation (Beermann, 2002; Birkelo, 2003) due to an increase in calpastatin activity (Hope-Jones, Strydom, Frylinck, & Webb, 2010). Consequently, the beneficial effects on muscle mass deposition can lead to changes in 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 quality 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, subprimal yield, and mRNA expression in heifers (Montgomery et al., 2009;

Leheska et al., 2009; Rathmann et al., 2012), and very few studies have reported the effects on *Bos indicus* cattle, such as Nellore (Cônsolo et al., 2015b).

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 (Vasconcelos et al., 2008; Scramlin et al., 2010). These carcass differences alter 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, subprimal cuts yield, meat quality, palatability traits, and mRNA expression in Nellore heifers.

2. 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).

2.1. 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

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São Paulo, in southeast Brazil from September 2012 to January 2013. The animals were in the feedlot for a total of 118 days, of which for the initial 85 days the animals were fed a common diet without ZH supplementation, followed by 30 days of ZH supplementation and 3 days of ZH withdrawal before slaughter.

2.2. Cattle

Seventy-two 18 months old Nellore heifers with an average body weight (BW) of 280 kg \pm 22 were housed in concrete collective pens (4 animals/pen; 3.0 m wide \times 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).

2.3. 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, 2000). 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. 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; 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 ration. Supplementation with ZH lasted for 30 days, with extra 3 days of ZH withdrawal before slaughter.

2.4. Slaughter and carcass samples

Subgroups of heifers were slaughtered at different time points to evaluate ZH effect on back fat, *Longissimus lumborum* (LL) area, and mRNA gene expression. Those measures were taken between the 12th and 13th ribs, across the LL, at the left half-carcasses. After 20 days of ZH supplementation (d 20), four heifers were randomly selected from each BW block (total of 12 heifers) for slaughter. Similarly, at the end of the experiment (d 33), all heifers were slaughtered, with 20 heifers (10 per treatment) being used to carcass deboning and collection of samples for meat quality evaluation.

Animals were slaughtered after 18 h fasting at the University of São Paulo experimental slaughterhouse, according to Sanitary and

Industrial Inspection Regulation for Animal Origin Products (Brasil, 1997). After 24 h postmortem, at the last slaughter, the subprimal cuts were weighed to measure the subprimal yields, according to a common industry practice employed in Brazil (Luchiaro Filho, 2000). The LL color was measured, immediately after cutting, at the level of the 12th thoracic vertebra using a Minolta CR 200b, according to the L*, a* and b* system (AMSA, 1995). In addition, four steaks were sampled (2.5 cm thick) from the LL of each animal, vacuum packed and aged for 0, 7, 14 or 21 days, and then frozen at -18 °C for analyses of cooking loss (CL), Warner Bratzler Shear Force (WBSF), and sensory quality.

2.5. WBSF and cooking loss analysis

Meat quality measurements of WBSF and CL were determined at the Laboratory of Animal Evaluation and Meat Quality of the University of São Paulo, using the methodology proposed by Wheeler, Shackelford, and Koohmaraie (2005) and previously described by Cônsolo et al. (2015a). 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 internal temperature of the steaks 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 fibers (Ferrari furadeira, São Paulo, SP, Brazil). Each core was sheared perpendicular to the muscle fiber 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.

2.6. 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), as previously described by Cônsolo et al. (2015a). Five sessions were performed, including three training sessions, one blank test, and one 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 °C and the internal temperature of the steaks was monitored individually. Each steak placed on a metal rack over an aluminum tray was turned over after reaching an internal temperature of 40 °C. Steaks were removed from the oven when reaching an internal temperature of 71 °C. 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, Civille, & Carr, 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).

2.7. Isolation of RNA and quantitative PCR

Longissimus lumborum samples were taken immediately after animal slaughter, 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

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

Item	% of DM
<i>Ingredient</i>	
Corn silage	35.0
Ground corn	50.4
Soybean meal	5.0
Wheat meal	6.0
Trace mineral mixture ^a	3.6
<i>Analyzed composition</i>	
CP	15.5
NDF	28.0
TDN ^b	75.1
NEg ^b , Mcal/kg DM	1.16

^a The trace mineral mixture contained (per kilogram) zinc, 728 mg; iron, 221 mg; fluorine (maximum), 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 (minimum), 109%; NPN (equivalent protein, maximum), 109%; monensin sodium, 1000 mg/kg.

^b Estimated according to NRC (2000).

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