Effect of beta-agonist in the activity of the major proteases involved in beef maturation and its impact on quality

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Abstract: Meat producers have adopted different strategies to maximize the production, yield, and quality of meat. Among them, the use of growth promoters as beta-agonists improves carcass performance for finishing beef cattle, increasing muscle growth and re- ducing the amount of fat gain. These compounds have been intensively studied during the past decades, but not all beta-agonists have been approved for use in meatproducing animals in several countries. Clenbuterol hydrochloride is used in several countries even though it is prohibited by international regulations. However, other alternatives, such as zilpaterol hydrochloride (ZH), which is considered as safe; increasing mRNA levels of myosin heavy chain-IIX (MHC-IIX). However, there are few studies on the effect of proteolytic activities involved in the maturation of the meat with ZH (Johnson, et al 2014; Zheng, et al 2018; Alvarado García, et al 2021).

Objectives: Determine the enzymatic activity of sarcoplasmic proteins (calpain, cathepsin, elastinase, and collagenase activities) and the presence of nebulin (myofibrillar protein) in beef with and without zilpaterol hydrochloride. As well as to evaluate the quality of meat with and without the supply of zilpaterol hydrochloride during wet and dry ripening using the Total Volatile Basic Nitrogen (TVB-N) method.

Materials and Methods: Twelve young whole male animals (Line F1, Cebu commercial cross with Swiss brown) with similar phenotypic characteristics were selected. Animals had a fattening period of 120 days. Six animals were supplied with zilpaterol hydro- chloride (ZH), and 6 animals were not provided with ZH in the diet were considered control. The *Longissimus dorsi* muscles were extracted (from the twelfth thoracic vertebra to the seventh lumbar) in both halves of the carcass. The samples were subjected to two maturation processes for 21 days: dry and wet, both at 4 °C, and taken at different storage time (0, 1, 3, 7, 14, and 21 days). The elastinolityc, collagenolytic and proteolytic activity of was determined using a specific substrate by fluorometric and spectro- photometric assays. The protein profile was characterized by electrophoretic separation using SDS-PAGE. The quality of the beef was determined using as an index of freshness the content of Total Volatile Basic Nitrogen (TVB-N).

Results and Discussion: The elastinolityc and collagenolytic activity showed an increase on day 3 of maturation, while collagenolyt- ic activity maintained this increase on day 7. However, in the following days of maturation, both activities decreased. These activi- ties were higher in beef with ZH and wet maturation than without ZH and dry maturation. These results suggest that the hardness of the meat is affected because this property of the meat is partly due to the connective tissue (collagen and elastin) that forms the endomysium and perimysium. Regarding cathepsin activity, this showed a decrease in beef with ZH and an increase in beef with- out ZH, all of these in both maturation methods. This impacts the reduction of collagenolytic activity in beef without ZH because cathepsin is associated with collagen degradation. The beef without ZH showed an increase in calpain activity from day 3 due to hydrolysis of the myofibrillar protein, mainly nebulin takes place. Therefore, nebulin presence had a decreased in beef with the same treatment. Otherwise, beef with ZH presented a reduction in calpain activity; this activity is influenced by the presence of nebulin in beef with ZH. The most significant proteolytic activity was observed in wet maturation. The TVB-N contents of beef with and without zilpaterol were below the suggested limit (30 mg Nitrogen/100g) in both maturation assays. The low contents of TVB-N represent a state of freshness since as these contents increase, they indicate an alteration in meat.

Conclusions: Determination of proteolytic activity (elastinolityc, collagenolytic, cathepsin, and calpains) demonstrate that the supply of ZH in bed exerts a significant effect compared to meat without ZH supply. The degradation of nebulin during maturation (wet and dry) is affected by the supply of ZH, as calpain activity decreases and the percentage of calpastatin inhibition increases. The quality of the meat, determined by TVB-N, is not affected by the supply of ZH during wet and dry maturation.

References: Johnson BJ, Smith SB, Chung KY. Historical Overview of the Effect of β-Adrenergic Agonists on Beef Cattle Produc- tion. Asian-Australas J Anim Sci. 2014 May;27(5):757-66. Zheng W, Leng X, Vinsky M, Li C, Jiang H. Association of body weight gain with muscle, fat, and liver expression levels of growth hormone receptor, insulin-like growth factor I, and beta-adrenergic re- ceptor mRNAs in steers. Domest Anim Endocrinol. 2018 Jul;64:31-37. Alvarado García, P.A. María Salud Rubio Lozano M.S., Sumano López H.S., Ocampo Camberos L., Tapia Pérez. G., Delgado Suárez E. Aguilar Acevedo J. Evaluation of two supplemen- tal zilpaterol hydrochloride sources on meat quality and carcass traits of crossbred Bos indicus bulls in the tropics. Rev. Mex. de Ciencias Pecuarias. 2021(1): Epub 20-Sep.

Key words: Beta-agonist, Zilpaterol, Elastinolityc, Collagenolytic, Muscle proteases