Meat Science 105 (2015) 63–67

Contents lists available at ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

Effect of an allostatic modulator on stress blood indicators and meat quality of commercial young bulls in Mexico

M.S. Rubio Lozano [a,*], R.D. Méndez Medina [b], K. Reyes Mayorga [a], M.E. Rubio García [a], M.A. Ovando [a], T.M. Ngapo [c], F.A. Galindo Maldonado [d]

[a] Meat Science Laboratory, Centro de Enseñanza Práctica e Investigación en Producción y Salud Animal, Facultad de Medicina Veterinaria y Zootecnia, Cruz Blanca 486, San Miguel Topilejo, Mexico DF 14500, Mexico
[b] Pathology Department, Facultad de Medicina Veterinaria y Zootecnia, Avenida Universidad 3000, Circuito Exterior, Ciudad Universitaria, Del. Coyoacán, Mexico DF 04150, Mexico
[c] Food Research and Development Centre, Agriculture and Agri-Food Canada, 3600 Boul. Casavant Ouest, Saint-Hyacinthe, Québec J2S 8E3, Canada
[d] Ethology and Wildlife Department, Facultad de Medicina Veterinaria y Zootecnia, Avenida Universidad 3000, Circuito Exterior, Ciudad Universitaria, Del. Coyoacán, Mexico DF 04150, Mexico

ARTICLE INFO

Article history:
Received 13 August 2014
Received in revised form 6 February 2015
Accepted 10 March 2015
Available online 19 March 2015

Keywords:
Allostasis
Allostatic modulator
Meat quality
Blood stress indicator
Beef

Chemical compounds studied in this article:
Ascorbic acid (PubChem CID: 54670067)
Acetoxybenzoic acid (PubChem CID: 2244)
Sodium chloride (PubChem CID: 5234)
Potassium chloride (PubChem CID: 4873)

ABSTRACT

To assess the effect of an allostatic modulator (AM) on stress blood indicators and meat quality traits, the feed of 80 non-castrated 18–20 month-old bulls was supplemented with 10 g/day of an AM for 30 days before slaughter. Another 80 bulls served as control animals. The AM was comprised of ascorbic acid, acetoxybenzoic acid and sodium and potassium chloride. Blood samples were taken at slaughter for analyses of hematocrit value, erythrocyte and leukocyte counts, and glucose, lactate and cortisol concentrations. Post-mortem measures of meat color and pH were made at 24 h and color, shear force and cooking loss on meat from 20 animals at 28 days. The AM supplementation resulted in lower hematocrit value, erythrocyte count and glucose level ($P < 0.05$), higher a* ($P < 0.0001$) and b* ($P < 0.0001$) at 24 h and lower b* ($P < 0.05$) at 28 days. Thus AM treatment improved some stress blood indicators and meat color and therefore merits further investigation.

1. Introduction

Improper and poor pre-slaughter handling of animals during production, transport, lairage and stunning can result in stress that directly affects animal welfare (Adzitey, 2011). In particular, Pérez Linares, Figueroa Saavedra, and Barreras Serrano (2006) showed that serious livestock welfare problems occur most often in the pre-slaughter period from transportation to slaughter. Cattle welfare concerns related to transportation and lairage include, but are not restricted to, limited access to feed and water, exposure to variable climatic conditions, noise and vibrations, poor handling and mixing with unfamiliar animals (Fazio & Ferlazzo, 2003; Schwartzkopf-Genswein et al., 2012). Increasing interest in animal welfare has stimulated research on pre-slaughter stress factors which in recent years have been widely studied to minimize or eliminate negative impacts on the animal's well-being (Adzitey, 2011), and as a number of studies have also shown that pre-slaughter stress can negatively impact carcass and meat quality, research interest is equally incited for economic reasons.

However, even with optimal handling, it is generally agreed that transport and pre-slaughter procedures are inherently stressful to livestock. While most of the studies to date have focused on measuring and minimizing the stressors, an alternative approach has been taken in Mexico aimed at helping the animal to cope. An allostatic modulator (AM) has been formulated as an anti-stress agent for inclusion in feed (Rubio-García, Castañeda-Serrano, Rubio-Lozano, & Ponce-Alquicira, 2013). Allostasis is the maintenance of physiological homeostasis during times of stress (McEwen & Wingfield, 2003) and allostatic load is how hard an individual must work in order to maintain that balance with allostatic overload occurring when the energy requirements to maintain the balance exceed the capacity of the animal (Herring & Gawlik, 2007). The proposed AM modulates the action of stress–response compounds triggered during pre-slaughter handling, including catecholamines, corticosterone, cortisol and hormones that cause protein catabolism.

* Corresponding author. Tel.: +52 55 5484 0515; fax: +52 55 5484 0514.
E-mail address: msalud@unam.mx (M.S. Rubio Lozano).

http://dx.doi.org/10.1016/j.meatsci.2015.03.012
0309-1740/

This safe, dietary drug is composed of acetoxybenzoic acid, ascorbic acid and sodium and potassium chloride. Acetoxybenzoic acid, also known as acetylsalicylic acid, irreversibly inhibits the cyclooxygenase pathway, which converts arachidonic acid to prostaglandins and thromboxanes (Di Luigi, Guidetti, Romanelli, Baldari, & Conte, 2001). Arachidonic acid metabolites play a role in the regulation of pituitary hormone secretion, including the release of ACTH (Nye et al., 1997), and are therefore important in reducing the negative effects of corticosteroids released during stress episodes.

Addition of dietary ascorbic acid to poultry prior to their preparation for slaughter, significantly reduced bird stress responses inhibiting the release of ACTH and adrenal gland enzymes, and decreasing the release of glucocorticoids and mineralocorticoids (Belge, Çinar, & Selçuk, 2003; Çinar et al., 2006; Satterlee, Aguilera-Quintana, Munn, & Krauttman, 1989).

Finally, ante-mortem physiological responses to stress include dehydration and electrolyte imbalances in muscle, which can affect both the animal's well-being and carcass and meat quality (Schaefer, Dubeski, Aalhus, & Tong, 2001). Although the major body fluid compartments differ in electrolyte composition, they are maintained in osmotic equilibrium. Sodium is the major determinant of extracellular and total body water, whereas potassium is the major determinant of intracellular fluid volume and both these cations are lost during ante-mortem stress (Schaefer et al., 2001). Schaefer, Jones, Tong, and Young (1990) suggested that shifts in major electrolytes, particularly chloride ion, sodium and potassium, appear to have a significant correlation with several meat quality traits. The provision of an electrolyte solution for consumption before slaughter contributed to more stable serum, tissue and urine electrolyte values which also contributed to an improved meat quality and carcass yield.

Preliminary studies incorporating the AM in poultry diets for 2 days prior to slaughter showed improvements in both animal welfare of the birds and quality of the ensuing meat (Rubio-García, Castañeda-Serrano, Rubio-Lozano, & Ponce-Alquicira, 2014b; Rubio-García, Rubio-Lozano, Ponce-Alquicira, Rosario-Cortes, & Castañeda-Serrano, 2014a; Rubio-García et al., 2013). A natural extension of this work is to investigate the impact of the dietary AM on other domestic animals exposed to stressful environments, such as beef during the transport and slaughter process. In Mexico, it is also of note that beef production is predominantly based on *Bos indicus* breeds, which are reported to be of a more excitable temperament than *Bos taurus* cattle (Hearnshaw & Morris, 1984). Therefore, the aim of this research was to determine the effect of the AM in the diet of finishing cattle on stress blood indicators and meat quality characteristics.

2. Materials and methods

2.1. Animals and treatments

The study was carried out in the spring of 2012 on a ranch (latitude 18°27′N, longitude 96°21′W, altitude 27 m) in the State of Veracruz, Mexico which has a tropical climate with temperatures varying from 18 to 34 °C. A total of 160 crossbred *B. indicus* non-castrated bulls were housed in two separate pens of 80 bulls each. The bulls were commercial crosses (Zebu × Charolais and Brown Swiss) of 20–24 months of age (about 450 kg). The pens were 30 m × 20 m and included a 20 m long feeder of approximately 0.5 m diameter and a 3 m × 1 m × 0.9 m water trough, had concrete non-slip floors and were covered by a 3 m high galvanized sheet metal roof with 30% shadow. Feed and water were provided ad libitum. The diet of group 1 was supplemented with the AM (equivalent of 10 g/animal/day) for the last 30 days of the finishing stage; the diet of group 2 was not supplemented with AM (control). Otherwise, handling of both groups was the same throughout the test period and during slaughter.

The AM was compounded from ascorbic acid (100 g), acetoxybenzoic acid (140 g) and sodium- (128 g) and potassium chloride (128 g). The AM (60 kg) was then added to ground corn (1940 kg) and mixed well to obtain a homogenous pre-mixture with an AM concentration in feed of 30 g/kg. A pre-mixture concentration of 25.3 kg/ton of feed was then used to achieve an average daily AM intake of 10 g/animal for the test period during which the average daily feed intake was 13.2 kg/animal.

At the end of the 30-day treatment the animals were transported 7.5 km over 25 min in commercial cattle trucks with three separate compartments (17, 18 and 5 animals/each) to a Federally Inspected Type (TIF) slaughterhouse. Two operators unloaded the animals at the slaughterhouse via a ramp (0.82 cm wide, 30% inclination angle, anti-slip floor and concrete walls) over a period of 18 min. Animals were kept in lairage (13–14 animals/pen) for 3.5 h before stunning and slaughter. Neither during transportation nor during lairage were unfamiliar animals mixed. In Mexico, TIF slaughterhouses comply with standard commercial practices for animal slaughter, including mechanical stunning.

2.2. Blood sampling

During exsanguination, blood samples were collected directly into sterile 10 ml Vacutainer tubes and placed on ice. Two samples were taken from each of 160 animals (80 control and 80 AM treated animals). Vacutainer tubes containing EDTA as an anticoagulant at a proportion of 2 mg/ml of blood were used for the analyses of blood cellular components (hematocrit value, hemoglobin content, erythrocyte and leukocyte counts), while Vacutainer tubes without anticoagulant were used for the analyses of blood biochemical components (glucose, lactate and cortisol). The blood for the analyses of the biochemical components was centrifuged at 2500 rpm for 10 min (room temperature) and the serum was separated into 5 ml sterile vials and stored at 0 °C until analysis. The samples used for the analyses of cellular components were stored at 4 °C until required. All samples were processed at the Laboratory of Clinical Pathology, Faculty of Veterinary Medicine of the National Autonomous University of Mexico.

2.3. Analyses of hematological and biochemical blood components

Hematological blood components were analyzed in blood from 53 animals (32 control and 21 AM treated animals); biochemical blood components were measured in the blood samples from all 160 animals. Hematocrit value (%) was obtained using the micro-hematocrit technique of Schalm, Jain, and Caroll (1975). Total erythrocyte and leukocyte counts were obtained by the method described by Nuñez and Bouda (2007). Leukocyte differential count and hemoglobin concentration were determined according to Schalm et al. (1975). Plasma glucose concentrations were determined using the GOD-PAP test without deproteinization (GL 2623, RANDOX®). To determine lactate concentration, a measurement technique based on an enzymatic UV test was applied. The method consists of a reaction to transform the lactate in pyruvate, which produces hydrogen peroxide (RANDOX® Manual RX MONZA). Plasma concentrations of cortisol (μg/dl) were determined using a competitive immunoassay technique (Munro & Stabenfeldt, 1984). The plates were read using a Dynatech-MRX Microplate Reader (DYNEX Technologies, Inc., VA, USA) at 410 nm.

2.4. Meat quality characteristics

At 24 h post-mortem, steaks were cut from all 160 carcasses at the 12th and 13th ribs of the *Longissimus dorsi* muscle (LD) for color and pH measures. Sites in the steaks without fat, bone or connective tissue were chosen for the meat pH and color measures. After 30 min blooming, color (CIE L*a*b*) was measured as the mean of duplicate readings using a portable HunterLab MiniScan XE Spectrophotometer (400–700 nm; D65 light source with 10° viewing angle; Model 4500 L, Hunter Associates Laboratory, Reston, West Virginia, USA). The pH was measured as