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Pre-slaughter administration of glycerol as carbohydrate precursor and osmotic agent to improve carcass and beef quality

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ABSTRACT

Glycerol is a by-product from biodiesel production. This molecule is a carbohydrate precursor, thus it maintains the pH of beef at below 5.8 after slaughter, contributing to the prevention of Dry Firm Dark (DFD) meat. Furthermore, glycerol osmotic properties could contribute to alleviating the effects of some stress situations. The aim of this study was to evaluate the effect of administering crude glycerol (2 g kg^{-1} body weight; via nasogastric tube or in drinking water) 24 h prior to the slaughter of young bulls, on plasma parameters, liver glycogen levels, carcass (body weight, dressing percentage, carcass weight and length), and beef quality (proximal composition, pH, colour, water holding capacity (WHC), drip loss, cooking loss, and sensory attributes). In general, the plasma parameters and liver glycogen were not affected by glycerol administration ($P > 0.05$). Transport had a significant effect on total protein, albumin, glucose, sodium, potassium, chloride, and lactate plasma levels. Carcass parameters, proximal composition, pH, colour and sensory attributes were not affected ($P > 0.05$) by glycerol treatment but WHC was ($P < 0.05$). The glycerol did not affect the stress markers in plasma during the transport, carcass or meat quality negatively, but it seems to improve WHC of beef.

1. Introduction

Recent interest in biodiesel as a renewable energy resource has led to the production of large amounts of glycerol as a by-product. Several studies have identified animal feed as a possible use for unrefined glycerol (Della Casa et al., 2009; Lammers et al., 2008; Parsons et al., 2009), since the molecule may be converted into glucose (Mach et al., 2009). Glycerol is converted to propionate in the rumen (Mach et al., 2009), which is transformed into glucose and provide energy through the gluconeogenic pathway, and then is converted into glycogen in the liver. Glycogen is used as a substrate for postmortem lactic acid production in muscle and therefore the pH falls (Immonen and Puolanne, 2000). So it can produce sufficient pH decrease to reach the target value (=below 5.8) after slaughter, contributing to the prevention of Dry Firm Dark (DFD) meat, which causes substantial economic losses in the beef meat mark (Brandon, 2004). Indeed, some Spanish meat industries penalise carcass price with discounts of 30% when meat pH measured 24 h after slaughter is greater than 5.8 (Mach et al., 2008a). Previously, it was studied the administration of other substances previous slaughter, e.g. sorbitol, to prevent the incidence of this defect on meat (Bignon, 1992). Glycerol supplementation might therefore be considered for preventing the pH of beef from exceeding 5.8. Furthermore, osmotic properties have been attributed to glycerol (Parker et al., 2007). Research in human has shown that glycerol included in sports soft drinks increases water retention in high-performance athletes (Anderson et al., 2001, Wagner, 1999). The ingestion of this type of drink before exercise results in hyperhydration of the body cells so, in a parallel way, it could maintain body water content in stress situations, such as long distance transport (Parker et al., 2007), by which contributes to prevent water losses in meat, while improving some quality attributes like juiciness and tenderness (Offer and Knight, 1988).

In *Bos indicus* steers, Parker et al. (2007) concluded that glycerol administration at 2 g kg^{-1} body weight (BW) before transport in a single dose, shows promise as a prophylactic treatment for attenuating the effects of long distance transportation by maintaining body water, decreasing the energy deficit and preserving health and muscle quality. However, the authors did not study the effect of glycerol on carcass or beef quality.

The hypothesis of this study was based on the glycerol role as carbohydrate precursor and osmotic molecule that could improve

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carcass and meat quality. Due to nasogastric tube administration is not a farm practice; administration in drinking water will be studied as an alternative. Higher levels of glucose in blood might be expected in the glycerol groups, because between 80% and 90% of glycerol is fermented in the rumen and provides propionate as a glucose precursor (Ramos and Kerley, 2012), so blood analyses were carried out. In addition, as glucose could be transformed into glycogen in liver and muscle, glycogen content was measured. Then, to evaluate the transport as stress situation, other parameters, as cortisol were studied. So, the aims of this research were to study the effect of pre-slaughter glycerol administration by nasogastric tube or in drinking water on plasma parameters, liver glycogen levels, carcass parameters and beef quality in young bulls.

2. Materials and methods

2.1. Animals and dietary treatments

All procedures involving animals were approved by the University of Murcia Ethics Committee, and the animal care and experimental procedures used in this study conform to national and EU regulations and guidelines (EU Directive 86/609, as modified by Directive 2003/65, OJEU, 2003), concerning the protection of animals used for scientific research. The crude glycerine obtained from biodiesel production had the following chemical composition: 86.6% glycerol, 7.50% moisture, 5.88% ash, 3.06% chloride, 2.00% sodium, 0.052% potassium, 0.004% calcium and 0.003% methanol (Abengoa Bioenergy, San Roque, Cádiz, Spain).

All bulls had been raised with the habitual management in Spanish intensive farm and the diet was consisted in concentrate and barley straw *ad libitum*. Forty-five Limousine bulls (625 ± 32.2 kg live body weight, mean $\pm$ s.d., BW and 16-18 months old), from the same animal herd were deprived of concentrate 12 h before transport, selected, weighed and randomly allocated to three different treatments (15 bulls per treatment) on the farm a week before of the experiment, Control, no supplemental glycerol; GNT, glycerol administered by nasogastric tube (7 mm o.d. $\times$ 5 mm i.d.) at 2 g kg^{-1} BW in a single dose, with 1440 g crude glycerine (1252 g of glycerol) and 500 mL of water per animal, 24 h before transport to abattoir,; and GW, glycerol administered in drinking water, 24 h before transport to abattoir, at same dose as in the GNT treatment, by adding 18 g of crude glycerine in 1000 mL of water, recording a water daily ingestion of 80 L per bull (all water was consumed). For that, a week before to glycerol administration, the daily water intake per animal was controlled during five consecutive days and a mean of 80 L per animal was observed. Thus, the crude glycerine intake was 1.44 kg per animal (2 g kg^{-1} BW of glycerol as GNT treatment).

The GW treatment consisted of a continuous supply of glycerol in drinking water during the 24 h prior transport to the abattoir to facilitate animal management in farm conditions. The animals were transported to a commercial abattoir, 30 km from farm. Slaughtering took place within 1 h of arrival. The animals were stunned by captive bolt pistol, suspended by their hind leg and exsanguinated. The bulls were slaughtered according Directive 1009/2009 (OJEU, 2009) and dressed using standard commercial procedures.

2.2. Blood sampling and measurement of variables

Blood samples were taken from the caudal vein of each bull before the treatments and after 30 min of arriving at the slaughterhouse (one 10 mL heparin tube, BD Vacutainer®, LH PST; Becton, Dickinson, Madrid, Spain), to compare the effect of glycerine administration and probe if the transport was a stress factor. Samples were immediately placed in ice-water until centrifugation at 1500g for 10 min. Plasma was decanted and frozen at -20 °C for determinations (total protein, albumin, glucose, cortisol, phosphorus, calcium, sodium, potassium, chloride, and lactate) in the Veterinary Hospital of the University of Murcia. Total protein, albumin, calcium, phophorus and glucose were analysed in an automated clinical chemistry analyser (Olympus AU2700; Olympus Diagnostica GmbH, Hamburg, Germany). Cortisol was analysed with the Immulite System (Siemens Health Diagnostics, Deerfield, IL, USA). Intra- and inter-assay coefficients of variation (CVs) were 7.3% and 10.5%, respectively. Sodium, potassium, chloride and lactate were analysed using an automated analyser (ABL system, Radiometer) using selective membrane electrodes.

2.3. Carcass traits

Following slaughter, liver samples (5 g) were taken and frozen in liquid nitrogen at -85 °C for glycogen analysis, as indicated by Dreiling et al. (1987). Carcasses were stored at 2 ± 1 °C for 24 h and then carcass weight (CW) and carcass length (CLe) were recorded and the dressing percentage (DP) was calculated. At this time, the pH (pH24) was measured with a portable pHmeter (Crison GLP21 equipment) with a penetrating electrode, in the loin, between the 12th and 13th ribs (ISO 2917, 1999). Then, the left *Longissimus dorsi* muscle was removed from T4 to T10, 30 cm length, of each animal and was vacuum-packaged and stored for six days at 4 °C for aging (Vitale et al., 2014). Aging beef improves sensory parameters, decreases hardness and drip loss and modifies fat composition (Ansejo et al., 2005).

Later, left loins of each animal were cut into 2 cm thick cuts and some were refrigerated (2 °C) for moisture, fat, colour, water holding capacity (WHC), drip losses (DL), and cooking losses (CL) analyses. The rest of the samples were vacuum-packaged (R-RE; Industrias RAELMA, S.L., Madrid, Spain) in bags (BB3050, Cryovac, Seward, U.K, oxigen transmission ratio 0.83 $cm^3\ m^{-2}\ h^{-1}$ in 24 h) and frozen (-18 °C) to be analysed later (protein, fatty acid profile, sensory analysis).

2.4. Meat quality

Instrumental meat quality was assessed in the *Longissimus dorsi* muscle. Meat pH seven days postmortem (pH7) was measured as previously described. The proximate composition (moisture, total protein and intramuscular fat content) was determined by AOAC (1990, 1999). Samples were bloomed for 20 min before colour measurements to ensure a stable data (Brewer et al., 2001) and then Redness (a^*), yellowness (b^*) and lightness (L^*) coordinates from C.I.E. L*a*b* space were measured on the surface of the loin using a CR-400 Chroma Meter (Minolta Ltd., Milton Keynes, Osaka, Japan) with a 8 mm-diameter aperture and using D65 illuminant. Three measurements were taken on each chop sample from each animal (total 45).

The WHC was determined following the pressing method described by Grau and Hamm (1953), and the results are expressed as a percentage of the initial sample weight. For DL, the samples (2 cm thick) were packaged in polystyrene trays with a supporting mesh ensuring that the sample does not make contact, covered with a permeable film (MICAL® professional, Miquel Alimentació Group S.A.U, Vilamalla, Spain) and stored at 4 °C (Honikel, 1998). The samples were weighed 0, 72 and 120 h post-packing. Drip losses are expressed as a percentage of the initial sample weight.

To evaluate the CL, the meat samples (two 2 cm thick chops *per* animal) were placed in open polyethylene bags and heated at 75 °C for 20 min in a water bath up to an internal temperature of 72 °C, as recommended by Vergara et al. (2003). Cooking loss was