

Relationship between glycerol administration to livestock 24 h before sacrifice and indicators of physiological and oxidative stress

Mariana Huerta-Jiménez • María Esther Ortega-Cerrilla • José Guadalupe Herrera-Haro • Jorge R Kawas-Garza • Antonio Díaz-Cruz • Cuauhtémoc Nava • David Hernandez-Sánchez • Eusebio Ortega-Jimenez • Alma Delia Alarcón-Rojo

Huerta-Jiménez M (Corresponding author) • **AD Alarcón-Rojo**

Facultad de Zootecnia y Ecología, Universidad Autónoma de Chihuahua. Periférico Francisco R. Almada Km 1. Chihuahua, Chihuahua, Mexico CP33820.

email: mhueraj@uach.mx

ME Ortega-Cerrilla • JG Herrera-Haro • D Hernández-Sánchez

Programa de Ganadería, Colegio de Postgraduados. Km 36,5 Carretera México-Texcoco, Montecillo Texcoco Edo de Mexico CP 56230.

JR Kawas-Garza

Facultad de Agronomía. Universidad Autónoma de Nuevo Leão, Avenida Francisco Villa S / N, Colonia Ex-fazenda El Canadá, Escobedo, Nuevo Leon, Mexico CP 66050.

A Díaz-Cruz • C Nava

Departamento de Nutrición Animal y Bioquímica, Universidade Nacional Autónoma de Mexico, Distrito Federal, México CP 3000.

E Ortega-Jiménez

Programa en Ciencias en Agroecosistemas Tropicales, Colegio de Postgrados, Campus Vera, Carretera Xalapa-Vera Km 88,5 Carretera Federal Xalapa-Vera CP 91700 Vera, Mexico.

Received: April 08, 2018 • Revised: June 04, 2018 • Accepted: June 04, 2018

Abstract The objective of this study was to evaluate the effect of oral administration of glycerol, as a source of energy, to steers 24 h before slaughter on biochemical indicators of physiological and oxidative stress. Fifty Zebu x Swiss- or Simmental-cross steers were selected at random at the finalizing stage impending slaughter. Blood samples were collected from the jugular vein before administering the treatments and 24 h afterward. After sacrifice, samples of the Longissimus dorsi muscle were collected. Biochemical indicators evaluated in bovine serum were hematocrit (VGA), lactate dehydrogenase (LDH), lactate (LAC), cortisol (COR), glucose (GLU), β-hydroxybutyrate (BHT), creatine kinase enzyme (CK), free non-esterified fatty acids (NEFA), antioxidant activity (FRAP), thiobarbituric acid reactive substances (TBARS), and glutathione peroxidase reaction (GSH-Px). The variables evaluated in meat were TBARS, FRAP, pH, texture and shelf life. Analysis of the stress biomarkers evaluated in bovine serum did not reveal differences ($P>0.05$) between treatments T0 and T1 for the variables COR, VGA, GLU, NEFA, BHT or CK. There were, however, differences between sampling times for the variables FRAP ($P = 0.021$) and GSH ($P = 0.006$). The indicators FRAP and TBARS in the sampled meat were not different ($P>0.05$) between treatments. Meat pH changed over time ($P=0.0002$), but not its texture ($P>0.05$). It is concluded that the administration of glycerol as an energy supplement 24 h before sacrifice did not modify the

balance of physiological constants of the evaluated steers, nor did it produce significant changes in shelf life of meat.

Keywords: carcass quality, stress biomarkers, ruminants

Introduction

Handling animals before and during slaughter causes stress and possible loss of homeostasis in the individual (Amtmann et al 2006). When the body uses fat reserves as a source of energy, glycerol, and fatty acids are released into the blood stream. Glycerol transforms into glucose in the liver and kidneys, providing energy for cell metabolism (Wang et al 2009a), reducing the imbalance of physiological and oxidative stress indicators. There is a study focused on the use of glycerol for therapeutic purposes in the treatment of ketosis in cows (Benitez and Giraldo 2012). Parker et al (2007) reported that the administration of glycerol to *Bos indicus* cattle in a single dose of 2 kg^{-1} LW before shipping, and concluded that the osmolyte glycerol 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. Another study showed the evaluated the effects of glycerin supplementation on performance, ruminal fermentation, metabolism, and carcass and meat quality in Holstein bulls fed high-concentrate diets. They observed that feeding concentrate containing up to 12.1% of glycerin does not lead

to detrimental effects on performance, ruminal fermentation, metabolism, and carcass and meat quality variables (Mach et al 2009).

A study conducted by Macarena et al (2015) showed that administering glycerol to Limousin bulls 24 h before slaughter through both a nasogastric tube and drinking water did not affect blood parameters, carcass quality, pH or sensorial meat quality. The present study posed the hypothesis that glycerol, as a gluconeogenic precursor, minimizes the effect of physiological and oxidative stress attributed to the increase in the efficiency of energy use of feeds by ruminants and that this effect can be measured by biochemical indicators of physiological and oxidative stress. We evaluated whether administering glycerol 24 h before slaughter counteracts the stress produced by handling during transport, and loading and unloading cattle before slaughter, maintaining the indicators of physiological stress within normal ranges to keep homeostasis in the animal and, after slaughter, the shelf life of the meat.

Materials and Methods

Field phase

Fifty male steers, Zebu crosses with Swiss or Simmental, were selected at random 24 h before slaughter. The steers were in the finalization stage, in intensive fattening, with an average body weight of 450 kg, and in the feedlot for 90 and 102 days on average. Two treatments were tested ($n= 25$ animals per treatment): T0= control (without glycerol) and T1= 600 mL glycerol (85 % pure) animal $^{-1}$. The treatments were administered orally with a Syrman graduated syringe. Animals were handled using a clamp to hold them. Blood samples were taken from the 50 animals before administering glycerol to determine basal parameters and 24 h after glycerol administration (at the moment of beheading in the slaughterhouse).

The samples were collected by puncturing the jugular vein with 5 mL vacutainer tubes containing EDTA anticoagulant. Hematocrit (VGA), lactate dehydrogenase (LDH), lactate (LAC), cortisol (COR), glucose (GLU), β -hydroxybutyrate (BHT), and free non-esterified fatty acids (NEFA) were quantified. Tubes with 10 mL EDTA were used to collect blood samples to evaluate antioxidant activity (FRAP), thiobarbituric acid reactive substances (TBARS), and the glutathione peroxidase reaction (GSH-Px). Samples used to determine the enzyme creatine kinase (CK) were collected in 5 mL vacutainer tubes without anticoagulant. The animals were shipped to the slaughter house at 5:30 am at an ambient temperature of 15 °C and 90 % humidity under fair handling conditions, avoiding the use of an electric prod. Transport lasted 30 minutes in a double-floored container with capacity for 40 steers. The steers were kept in a reception

corral 4 to 5 h on average before the slaughtering process began.

Laboratory phase

After collection, the blood samples were refrigerated at 4 °C and centrifuged at 3,500 rpm for 10 min to obtain the serum (Power Spin™ Mx Centrifuge). The serum was then distributed in 2 mL Eppendorf tubes and stored at -40 °C until processing, except for the samples taken for determination of VGA, which were processed in the laboratory of the commercial ranch using the microcentrifugation technique (Micro-Hematocrit Centrifuge Model KHT-400) and capillary tubes (Fisher brand/ Micro-Hematocrit cat. No: 22-362-574). The serums were transported under refrigeration at 4°C to determine the biomarkers of physiological stress: GLU, LDH, LAC, BHT, NEFA and CK. For this determination, an atomic absorption spectrophotometer (Perkin Elmer) was used. COR was measured using the ELISA technique (Enzyme-Linked ImmunoSorbent Assay) with rabbit antibodies and horseradish peroxidase as the antigen. Readings were taken at a wavelength of 410 nm. FRAP, TBARS and GSH-Px were assessed in the serums. All the samples were processed in duplicate.

FRAP (Ferric Reducing/Antioxidant Power) was determined following the protocol of Benzie and Strain (1999, 1996) and using TPTZ chromogen and the antioxidant standard TROLOX. Readings were done with a spectrophotometer at 593 nm. The thiobarbituric acid reaction test was conducted according to the protocols of Botsoglou et al. (1994) and Gutteridge (1975). Glutathione peroxidase activity was determined by measuring the concentration of NADPH at 340 nm, following the procedure described by Lawrence and Burk (1976).

After slaughter, the carcasses were stored in refrigerated chambers at a temperature of 0 °C and, after 24 h, transported in refrigeration trucks to the boning room of the packing plant, where the second part of the sampling was done. This part consisted of collecting 500 g of the *Longissimus dorsi* muscle without bone at the 10th and 13th rib of 10 steers of each treatment. Once the samples of muscle were collected, they were vacuum packed and stored under refrigeration at 4 °C to evaluate shelf life at 7, 14, 21 and 28 d. FRAP (Benzie and Strain 1999, 1996) and TBARS (Botsoglou et al. 1994; Gutteridge, 1975) were then determined in homogenized beef at different times of maturation (7, 14, 21 and 28 days). pH and texture were also recorded. pH was determined with the method of meat homogenization and the reading was done with a potentiometer Pelican 1400 Case, Orion Research Model SA 210 (AOAC 2000). For texture analysis (TPA), the process was conducted using a Warner-Bratzler knife and the protocol of the texturometer TA.TX plus (Stable Micro Systems).

Statistical analysis

The variables evaluated in blood serum were analyzed as a completely random design with repeated measures and the PROC MIXED of SAS (Statistical Analysis System, version for Windows 9.0, 2000). The model for the analysis included the principal effects of treatment, time and the interaction treatment*time. For the variables assessed in meat (FRAP, TBARS, pH and texture), the procedure was similar. Means were compared with the Tukey test. The covariance structure appropriate for each variable was the component of symmetry (CS). The model used was the following:

$$Y_{ijkl} = \mu + T_i + \delta_{j(i)} + P_l + (TP)_{ik} + \varepsilon_{ijkl}$$

where: Y_{ijkl} = response variable in observation k, repetition j, treatment i; μ = general mean; T_i = effect of the i^{th} treatment ($i=1,2$); $\delta_{j(i)}$ = random error associated with the j^{th} animal (subject) within the i^{th} treatment; P_l = effect of the l^{th} period ($l=1,2,\dots,4$); (TP) = Interaction treatment*period; ε_{ijkl} = Random error associated with the k^{th} repeated measure within the j^{th} animal.

Results

The analysis of physiological stress biomarkers reported as indicative of acute stress revealed no differences ($P>0.05$) in CORT, VGA, GLU, NEFA, BHT or CK between the treatments, but LDH ($P=0.021$) and LACT ($P=0.001$) were different (Table 1).

There were differences in the effect of time ($P<0.05$) for the variables VGA, NEFA, BHT, CK, LACT and GLU. The interaction treatment*time was significant ($P=0.011$) only for GLU (Table 1).

Oxidative stress biomarkers in blood samples were different ($P>0.05$) between sampling times (Table 2) for the variables FRAP ($P=0.0209$) and GSH ($P=0.006$).

No differences ($P>0.05$) between treatments were observed in FRAP in meat, but differences ($P<0.05$) were found among the different lengths of shelf life. Interaction ($P=0.024$) between treatments and times was seen in the determination of TBARS, measured as an index of lipoperoxidation, which evidenced higher concentrations with the glycerol treatment as shelf life time increased (Table 3).

The decrease in pH during shelf life was significant ($P=0.0002$); however, no differences were observed between treatments nor in the interaction treatment*time (Table 3). The pH values for day 7 were $T_0=5.58$ and $T_1=5.54$, for day 14 $T_0=5.59$ and $T_1=5.60$, for day 21 $T_0=5.57$ and $T_1=5.53$ and day 28 $T_0=5.50$, $T_1=5.46$.

Finally, the results of texture during the times evaluated of shelf life were not significant ($P>0.05$). Thus, the texture values were considered tender meat, the cutting force

varying at 7 d ($T_0=2.068$, $T_1=1.878 \text{ kg cm}^{-2}$), 14 d ($T_0=1.723$, $T_1=1.846 \text{ kg cm}^{-2}$), 21 d ($T_0=1.884$, $T_1=2.023 \text{ kg cm}^{-2}$) and 28 d ($T_0=1.76$, $T_1=2.07 \text{ kg cm}^{-2}$) (Table 3).

Discussion

Studies with pigs (Madrid et al 2013), chicken (Kim et al 2013) dairy cows (Werner Omazic et al 2013), sheep (Volpi-Lagreca and Duckett 2017), goats (Chanjula et al 2014) and buffalo (Saleem et al 2018) on the effects of glycerol on carcass yield, milk quality, and nutrient digestibility have been published. In general, those studies reported improvement in nutrient utilization of the diet. Macarena et al (2015) observed that with glycerol supplementation before shipping, there was an increase in glucose concentration ($P<0.001$) after shipping in all the groups evaluated. In a study (Parker et al 2007) in which glycerol was administered to steers through a nasogastric tube before prolonged transport (48 h), higher levels of blood glucose ($P<0.001$) were observed 24 and 48 h after shipping in groups that received glycerol, compared to control groups (not shipped and with no glycerol administration). The results of the glucose levels in this study were not different between treatments, but they were different between sampling times ($P<0.05$) and with the interaction treatment*time. The differences between in this study and that reported by Macarena et al (2015) and Parker et al (2007) were due to the different shipping times and handling. In the case of our study, transport time from the ranch to the slaughterhouse was less than an hour, while steers were transported 24 and 48 h, respectively in the other studies. It is important to point out that in our study, the glucose levels found were below the concentrations ($T_0=1.588$ and $T_1=1.663 \text{ mmol L}^{-1}$) considered normal ($3.0-4.4 \text{ mmol L}^{-1}$). Probably as a consequence of an insulin response to increased glucose absorption from the small intestine and because the exogenous glucose supply caused a decrease in endogenous glucose synthesis. Moreover, Glycerol that is available to rumen microbes will be converted to propionic and butyric acids. The fraction converted to butyrate is metabolized by the ruminal epithelium, thus glycerol that is fed is actually ketogenic rather than glucogenic.

These results coincide with Knowles (2000) concerning the low levels of glucose that can occur during the 24 h after handling the animals during transport to the slaughterhouse, mainly because animals are recovering and not because of stressful situations. Also, Cunningham (1999) mentions that short fasting periods (4 to 6 h) produce hypoglycemia, which acts as a catecholamine-releasing factor and favors glycolysis and gluconeogenesis, which might be the cause of the low glucose levels found in this study. Socreppa et al (2017) evaluated the effect of different levels of crude glycerine (810.9 g glycerol/kg) replacing dry ground maize on intake, digestibility, microbial nitrogen (N) synthesis and N utilization in grazing beef cattle. They concluded that partial or total replacement of dry ground maize by crude glycerine in protein-energy supplements for grazing beef cattle exerted no changes on pasture intake, digestibility and N utilization. Therefore, the use of crude glycerine as an energy source in supplements for grazing cattle can be recommended. Similarly Granja-Salcedo et al (2017) determined whether a combination of crude glycerin and soyabean oil could be used

to partially replace maize in the diet of Nellore steers while maintaining optimum feed utilisation. They found that crude glycerine associated with lipids could be an energy source, which is a useful strategy for the partial replacement of maize in cattle diets, and that it could result in reduced total N excretion and ruminal methanogens without affecting intake and digestibility. The effect of ambient temperature and dietary glycerol supplementation on growth performance in beef cattle was studied by Kang et al (2017) and observed that the hot temperature month did not affect growth performance, but glycerol supplementation improved growth performance in a barn trial. In a feeding trial in metabolic cages with a temperature-controlled room, hot temperature decreased growth performance, but glycerol supplementation did not affect it.

As far as dairy cow concerns, the supplementation of glycerol has also shown that glycerol is a suitable replacement for corn grain in diets for lactating dairy cattle and that it may be included in rations to a level of at least 15% of dry matter without adverse effects on milk production or milk composition (Donkin 2008). Another study (Wang et al 2009b) showed that although milk yield and feed intake were not affected, glycerol-supplemented cows have a more positive energy status, suggesting that net energy availability may have been increased. On the other hand, Coskun et al (2012) did not observe a significant effect on milk yield, body weight and plasma glucose, nonesterified fatty acids and beta-hydroxybutyrate concentrations, but they pointed out that in the last day of study, milk solid non fat were higher and milk urea-N content were lower in pelleted feed+ glycerol group ($P < 0.05$), and Karami-Shabankareh et al (2013) demonstrated that feeding dry glycerol as a glucogenic supply may be useful to improve negative energy balance and reproductive efficiency in young cows which calve with high requirement of energy.

DeFrain et al (2004) reported that cows supplemented with an 860 g d⁻¹ dosage of glycerol during a period of 7 to 21 days during lactation reduced blood glucose levels and increased BHT blood levels. These results are similar to those obtained in this study and could be because glycerol was administered 24 h before transport and could have metabolized before the second evaluation.

Behaviour is a critical component used to evaluate the animals' wellbeing and it has been reported to have an effect on product quality. Šimová et al (2016) mentioned that pre-transport phase includes many aspects, such as on-farm handling, rearing conditions, assembly of animals, classifying, weighing, repenning in a new environment, regrouping, mixing with unfamiliar animals, and handling at loading, which is regarded as the most significant factor affecting animal welfare. Stress activates the animals' hypothalamic-pituitary-adrenal activity, triggering release of various stress hormones such as catecholamines and cortisol, thus glycogen depletion prior slaughter, elevated ultimate pH and poor muscle-meat conversion.

Recently, Saleem and Singer (2018) conducted two experiments to evaluate the effects of replacing corn with an increasing concentration of high-purity glycerol (>99%) on growth performance, economical efficiency, blood constituents and nutrient digestibility of growing lambs. They reported that glycerol supplementation had no effect ($P > 0.05$) on organic matter and CP digestion, but improved DM, crude fiber, ether

extract and nitrogen-free extract digestion. They concluded that glycerol can replace corn up to 10% of DM in the diets of growing lambs.

Other biomarkers evaluated in this study were CORT, VGA, NEFA, and BHT, which were within normal ranges for bovines, according to Knowles and Warris (2000) and Romero et al (2011). However, it has also been pointed out that even on long trips, the effect of transport can be minimal only if handling is done correctly (María et al 2003). This might explain why in the present study no differences were observed in steers supplemented with a single dose of glycerol since both, the feedlot personnel and the shippers, who loaded and unloaded the animals had good training and good livestock handling practices.

The CK concentrations ($T_0 = 662.19$ and $T_1 = 603.20$ U L⁻¹) were above the normal range (35 – 280 U L⁻¹), possibly owing to the physical activity of the animal. There were no differences, however, between treatments because the creatine kinase enzyme increases when the animal exercises more than is usual, a situation that coincides with that reported by Knowles and Warris (2000) and Averós et al (2008). Likewise, Romero et al (2011) mention that the basal levels of CK can increase due to fasting and exercise, in this study the conditions of fasting and fatigue of the animals to be transported before slaughter, are factors that can explain the difference between the times of evaluation that were observed for the CK variable. The results of the LDH ($T_1 = 3436.52^a$, $T_0 = 2742.38^b$) and LAC ($T_1 = 10.541^a$, $T_0 = 7.745^b$) concentrations in the two treatments were different. In both cases, higher concentrations were found in animals that received the glycerol treatment. Significant ($P=0.001$) differences in LDH ($P=0.021$) and LAC were found between treatments and times ($P=0.004$). LAC and LDH concentrations are related to muscular damage. However, in our study, the increment at the serum level was not indicative of muscular damage, but rather of physiological adaptation to routine handling.

Njisane and Muchenje (2017) have reported that pre-slaughter stress sometimes results to cattle attaining bruises, resulting to the affected parts of the carcass being trimmed and condemned for human consumption, downgrading of the carcass and thus profit losses.

It has been pointed out by Damtew et al (2018) that stressful conditions can reduce the fitness of an animal, which can be expressed through failure to achieve reproductive and production performance standards, or through morbidity and mortality. A treatment to mitigate the negative effect of long transport stress on cattle physiology, could include remedial strategies such as administration of vitamins, vaccines and feeding high energy diets, and electrolyte therapy should be considered.

In countries such as New Zealand the handling of animals is a topic of great importance. This year (2018) regulations have been reviewed and they established great fines to those persons who fails to comply with the regulation (Animal Welfare (Care and Procedures) Regulations 2018).

Moreover, information on the acute stress of livestock during handling exists but little has been done on shelf life or sensorial tests (María et al 2003). For this reason, it was important in this study to evaluate antioxidant states using the FRAP components. These components increased with the storage times evaluated; this is a normal process by the effect of refrigeration. The differences reported for the

Table 1 Mean and standard error of the mean (SEM) of the parameters indicators of physiological stress by effect of administration of glycerol 24 h before slaughter.

Variable	Treatments		SEM		Time		SEM		P > 0.05		
	T0	T1	T0	T1	1	2	1	2	Trat	Time	Trat*Time
COR (ng ml ⁻¹)	2.416 ^a	2.944 ^a	0.347	0.296	2.303	3.057	0.319	0.367	0.252	0.150	0.071
VGA (%)	37.810 ^a	38.020 ^a	0.679	0.602	36.540	39.290	0.552	0.635	0.820	0.0008	0.830
GLU (mmol L ⁻¹)	1.588 ^a	1.663 ^a	0.148	0.127	1.370	1.881	0.131	0.152	0.702	0.017	0.011
NEFA (mmol L ⁻¹)	0.339 ^a	0.318 ^a	0.023	0.212	0.238	0.419	0.018	0.020	0.515	<.0001	0.058
BHT (mmol L ⁻¹)	0.334 ^a	0.334 ^a	0.016	0.013	0.278	0.390	0.015	0.017	0.990	0.0001	0.525
LDH (U L ⁻¹)	2742.38 ^b	3436.52 ^a	222.60	188.10	2931.00	3247.9	210.500	241.830	0.021	0.366	0.797
CK (U L ⁻¹)	662.19 ^a	603.20 ^a	60.770	51.028	440.22	825.17	59.115	67.703	0.460	0.0003	0.113
LAC (mmol L ⁻¹)	7.745 ^b	10.541 ^a	0.602	0.526	7.709	10.577	0.507	0.585	0.001	0.0004	0.789

TRAT= Treatment; T0= Treatment without glycerol; T1= Treatment with glycerol; SEM= Standard error of the mean; COR= cortisol; VGA= hematocrit; GLU= glucose; NEFA= free non-esterified fatty acids; BHT= β -hydroxybutyrate; LDH= lactate dehydrogenase; CK= creatine kinase; LAC= lactate.

^{a,b}Different letters in the same row indicate significant differences ($P < 0.05$).

Table 2 Mean and standard error of the mean (SEM) of the parameters indicators of oxidative stress by effect of administration of glycerol 24 h before slaughter.

Variables	Treatments		SEM		Time		SEM		P>0.05		
	0	1	0	1	1	2	1	2	Trat	Time	Trat*Time
FRAP	0.329 ^a	0.354 ^a	0.135	0.115	0.318	0.366	0.012	0.014	0.163	0.021	0.571
TBARS	6.599 ^a	9.327 ^a	1.126	0.980	9.224	6.703	0.962	1.111	0.073	0.087	0.213
GSH ($\mu\text{mol NADPH min}^{-1}\text{L}^{-1}$)	3.353 ^a	3.141 ^a	0.192	0.162	3.675	2.818	0.181	0.208	0.404	0.006	0.843

FRAP= antioxidant activity; TBARS= thiobarbituric acid reactive substances; GSH= glutathione peroxidase reaction.

^{a,b} Different letters in the same row indicate significant differences (P< 0.05).**Table 3** Mean and standard error of the mean (SEM) of the effect of administrating glycerol 24 h before slaughter over antioxidant activity (FRAP), thiobarbituric acid reactive substances (TBARS), pH and texture to evaluate shelf life on days 7,14, 21 and 28.

Variables	Shelf life (Days)										P>0.05		
	7		14		21		28		SEM				
	T0	T1	T0	T1	T0	T1	T0	T1	T0	T1	Trat	Time	Trat*Time
FRAP	0.273 ^a	0.233 ^a	0.456 ^a	0.468 ^a	0.784 ^a	0.747 ^a	0.760 ^a	0.758 ^a	0.027 ^a	0.027	0.671	<.0001	0.940
TBARS	0.019 ^b	0.022 ^a	0.052 ^b	0.054 ^a	0.066 ^b	0.083 ^a	0.057 ^b	0.092 ^a	0.003	0.003	0.005	<.0001	0.024
pH	5.58 ^a	5.54 ^a	5.59 ^a	5.60 ^a	5.57 ^a	5.53 ^a	5.50 ^a	5.46 ^a	0.018	0.018	0.303	0.0002	0.815
Texture (g cm^{-2})	2068.7 ^a	1878.84 ^a	1723.43 ^a	1846.50 ^a	1883.93 ^a	2023.47 ^a	1759.30 ^a	2068.71 ^a	67.25	67.25	0.300	0.400	0.200

^{a,b} Different letters in the same row indicate significant differences (P< 0.05).

TBARS concentrations coincide with Soohyun et al (2015) in Korean Hanwoo cattle (*Bos taurus coreanae*), in which higher TBARS concentrations were observed due to storage times ($P<0.001$) and its interaction with animal age ($P<0.001$). In the same way, Xiong et al (2007) observed significant differences in TBARS in vacuum-packed beef stored under refrigeration below 3 °C. This process can be explained by the high content of unsaturated fatty acids and the presence of myoglobin, which favors lipid oxidation in raw meat during storage and refrigeration (Monahan 2000). These results are also corroborated by the results of Chan et al (1997), who evaluated lipid oxidation in meat and deduced that the increase in TBARS might be due to high concentrations of myoglobin. In our study, oxidative deterioration of lipids was significant despite the increase in FRAP, an indicator of exogenous antioxidant capacity. This indicator was significant over time ($P<0.05$) due to storage of the meat and the gradual increase in the storage times, 7 to 28 d, under refrigeration at 5 °C and to the processes of maturation and not by the effect of glycerol supplementation.

pH descended in a normal way due to the conversion of glycogen to lactic acid in an anaerobic medium, such as that in vacuum packing, causing pH to change as a normal process of constant degradation of organic matter. Average post mortem pH of beef is 5.4-5.8. Gregory (1998) mentions that the increase in physiological stress or physical activity in farm animals during shipping and *ante mortem* handling leads to exhaustion of muscular glycogen reserves before slaughter, which can result in a high final pH in the meat and tougher meat. Under such conditions beef can have a pH higher than 6.2, this meat has great water retention but it deteriorates soon at temperatures above freezing. In the present study, pH was not affected by glycerol supplementation nor by handling the steers before slaughter.

According to Miranda et al. (2009), the biphasic relationship between tenderness and final pH occurs because the proteolytic activity is lower with pH values of 6.3-5.8. In our study, final pH on day 28 of storage was below 5.8, indicating that the physical-chemical changes in the meat were favored by proteolytic degradation, changes in pH and increase in lactic acid during maturation (Young et al 2004).

Another study shows the effects of glycerin supplementation on performance, ruminal fermentation, metabolism, and carcass and meat quality in Holstein bulls fed high-concentrate diets (Mach et al 2009). The authors observed that feeding concentrate containing up to 12.1% of glycerin does not lead to detrimental effects on performance, ruminal fermentation, metabolism, and carcass and meat quality variables.

As it has been seen in the present study there is no consistent association between the stress parameters and meat quality measurements. Therefore, it is important to determine the appropriate parameters for assessing the level of stress.

Conclusions

Oral administration of 600 mL glycerol as a source of energy for fattening livestock 24 h before slaughter did not cause differences between treatments because glycerol is absorbed rapidly and only a single dose was administered before slaughter to evaluate its effect. The animals were not

under stress during handling and physical activity was minimal during transport of less than one hour. These conditions permitted maintaining some of the physiological and oxidative stress indicators within normal ranges without affecting meat shelf life.

Acknowledgements

The authors thank the Consejo Nacional de Ciencia y Tecnología for granting scholarship N° 311965 for doctoral studies and to LPI-7 of the Colegio de Postgraduados for partial funding of this research.

References

- (LI 2018/50) Association of Official Analytical Chemistry (2000) Official methods of analysis 17ed. AOAC. M.D. USA.
- Amtmann VA, Gallo C, Van Schaik G, Tadich N (2006) Relationships between ante-mortem handling, blood based stress indicators and carcass pH in steers. Archivos de Medicina Veterinaria 38:259-264.
- Animal Welfare (Care and Procedures) Regulations (2018) Wellington, New Zealand. Government of New Zealand.
- Averós X, Martín S, Riu M, Serratosa J, Gosálvez LF (2008) Stress response of extensively young being transported to growing-finishing farms under Spanish summer commercial conditions. Livestock Science 119:174-182.
- Benítez HS and Giraldo VLA (2012) Suplementación con glicerol crudo en ganado de leche: Co-producto de biodiesel evaluado in vitro y en la producción y calidad de la leche en el trópico alto de Antioquia, Colombia (Spanish edition). Editorial Academia Española.
- Benzie IF and Strain JJ (1996) The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Analytical Biochemistry 239:70-76.
- Benzie IF and Strain JJ (1999) Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Methods in Enzymology 299:15-27.
- Botsoglou NA, Fletouris DJ, Papageorgiou JE, Vassilopoulos VN, Mantis AJ, Trakatellis AG (1994) Rapid, sensitive and specific thiobarbituric acid method for measuring lipid peroxidation in animal tissue, food and feedstuff. Journal of Agricultural and Food Chemistry 42:1931-1937.
- Chan WKM, Faustman C and Decker E (1997) Lipid oxidation induced by oxymyoglobin with involvement of H₂O₂. Meat Science 46:181-190.
- Chanjula P, Pakdeechanuan P, Wattanasit S (2014) Effects of dietary crude glycerin supplementation on nutrient digestibility, ruminal fermentation, blood metabolites, and nitrogen balance of goats. Asian-Australasian Journal of Animal Sciences 27:365-374.
- Coskun B, Inal F, Gurbuz E, Polat ES, Alatas MS (2012) The effects of additional glycerol in different feed form on dairy cows. Kafkas Universitesi Veteriner Fakultesi Dergisi 18:115-120.
- Cunningham JG (1999) Textbook of Veterinary Physiology. 2^a ed. Ed. Interamericana McGraw-Hill. México.

- Damtew A, Yidersal Erega Y, Ebrahim H, Tsegaye S, Msigie D (2018) The effect of long distance transportation stress on cattle: a review. *Biomedical Journal Scientific & Technical Research* 3:1-5.
- DeFrain JM, Hippen AR, Kalscheur KF and Jardon PW (2004) Feeding glycerol to transition dairy cows: Effects on blood metabolites and lactation performance. *Journal Dairy Science* 87:4195-4206.
- Donkin SS (2008) Glycerol from biodiesel production: the new corn for dairy cattle. *Revista Brasileira de Zootecnia* 37:280-286.
- Granja-Salcedo YT, Messana JD, de Souza VC, Dias AVL, Kishi LT, Rebelo LR, Berchielli TT (2017) Effects of partial replacement of maize in the diet with crude glycerin and/or soyabean oil on ruminal fermentation and microbial population in Nellore steers. *British Journal of Nutrition* 118:651-660.
- Gregory NG (1998) Animal Welfare and Meat Science (Chapter 2 and 4). UK: CABI Publishing.
- Gutteridge JM (1975) The use of standards for Malonyldialdehyde. *Analytical Biochemistry* 69:518-526.
- Kang HJ, Piao MY, Kim HJ, Baik M (2017) Effects of ambient temperature and glycerol supplementation on growth performance in Korean cattle steers. *Journal of Animal Science* 95:298.
- Karami-Shabankareh H, Kafilzadeh F, Piri V, Mohammadi H (2013) Effects of Feeding Dry Glycerol to Primiparous Holstein Dairy Cows on Follicular Development, Reproductive Performance and Metabolic Parameters Related to Fertility during the Early Post-partum Period. *Reproduction in Domestic Animals* 48:945-953.
- Kim JH, Seo S, Kim CH, Kim JW, Lee BB, Lee Gil, Shin HS, Kim MC, Kil DY (2013) Effect of dietary supplementation of crude glycerol or tallow on intestinal transit time and utilization of energy and nutrients in diets fed to broiler chickens. *Livestock Science* 154:165-168.
- Knowles TG and Warriss PD (2000) Stress physiology of animals during transport. In: *Livestock Handling Transport* 2nd edition. Grandin T. (Ed). CABI International. New York.
- Lawrence RA, Burk RF (1976) Gluthatione peroxidasa activity in selenium deficient rat liver. *Biochemical Biophysical Research Communications* 71:952-958.
- Macarena E, Linares MB, Hernández F, Madrid J, Garrido MD (2015) Pre-slaughter administration of glycerol as carbohydrate precursor and osmotic agent to improve carcass and beef quality. *Livestock Science* 182:1-7.
- Mach N, Bach A, Devant M (2009) Effects of crude glycerin supplementation on performance and meat quality of Holstein bulls fed high-concentrate diets. *Journal of Animal Science* 87:632-638.
- Madrid J, Villodre C, Valera L, Orengo J, Martínez S, López MJ, Megías MD, Hernández F (2013) Effect of crude glycerin on feed manufacturing, growth performance, plasma metabolites, and nutrient digestibility of growing-finishing pigs. *Journal of Animal Science* 91:3788-3795.
- María GA, Villaruel M, Sañudo C, Olleta JL, Gebresenbet G (2003) Effect of transport time and ageing on aspects of beef quality. *Meat Science* 65:1335-1340.
- Miranda-de la Lama GC, Villaruel M, Olleta JL, Alierta S, Sañudo C and María GA (2009) Effect of the pre-slaughter logistic chain on meat quality of lambs. *Meat Science* 83:604-609.
- Monahan FJ (2000) Oxidation of lipids in muscle foods: Fundamental and applied concerns. In: E.A. Decker C Faustman and CJ Lopez-Bote (Eds.), *Antioxidants in muscle foods: Nutritional strategies to improve quality*. New York: John Wiley and Sons, Inc.
- Njisane YZ and Muchenje V (2017) Farm to abattoir conditions, animal factors and their subsequent effects on cattle behavioural responses and beef quality — A review. *Asian-Australasian Journal Animal Science* 30:755-764.
- Parker AJ, Dobson GP and Fitzpatrick LA (2007) Physiological and metabolic effects of prophylactic treatment with the osmolytes glycerol and betaine on *Bos indicus* steers during long duration transportation. *Journal Animal Science* 85:2916-2923.
- Romero PHM, Uribe-Velásquez FL and Sánchez VJ (2011) Stress biomarkers as indicators of animal welfare in cattle beef farming. *Biosalud* 10:71-87.
- Saleem A.M, Zanouny AI, Singar AM (2018) Effect of glycerol supplementation during early lactation on milk yield, milk composition, nutrient digestibility and blood metabolites of dairy buffaloes. *Animal* 12:757-763.
- Saleem, A.M and Singer AM (2018) Growth performance and digestion of growing lambs fed diets supplemented with glycerol. *Animal* 12:959-963.
- SAS, Statistical Analysis System for Windows 9 (2000) Institute Cary, NC, USA.
- Šimová V, Večerek V, Passantino A, Voslarova E (2016) Pre-transport factors affecting the welfare of cattle during road transport for slaughter - A review. *Acta Veterinaria Brno* 85:303-318.
- Socreppa LM, Moraes KAK, Oliveira AS, Batista ED, Drosghic LCAB, Botini LA, Paula DC, Stinguel H, Bento FC, Moraes EHBK (2017) Crude glycerine as an alternative energy feedstuff for beef cattle grazing tropical pasture. *Journal of Agricultural Science* 155:1-8.
- Soohyun Cho, Geunho K, Pil-Nam S, Beomyoung P and Sun Moon, K (2015) Effect of slaughter age on the antioxidant enzyme activity, color, and oxidative stability of Korean Hanwoo (*Bos taurus coreanae*) cow beef. *Meat Science* 108:44-49.
- Volpi-Lagreca G, and Duckett SK (2017) Supplementation of glycerol or fructose via drinking water to grazing lambs on tissue glycogen level and lipogenesis. *Journal of Animal Science* 95:2558-2575.
- Wang C, Liu Q, Huo WJ, Yang WZ, Dong KH, Huang YX and Guo G (2009a) Effects of glycerol on rumen fermentation, urinary excretion of purine derivatives and feed digestibility in steers. *Livestock Science* 121:15-20.
- Wang C, Liu Q, Yang WZ, Yang XM, Huo WJ, Dong KH, Huang YX, He DC (2009b) Effects of glycerol on lactation performance, energy balance and metabolites in early lactation Holstein dairy cows. *Animal Feed Science and Technology* 151:12-20.
- Werner Omazic A, Tråvén M, Bertilsson J, Holtenius K (2013) High-and low-purity glycerine supplementation to dairy cows in early lactation: effects on silage intake, milk production and metabolism. *Animal* 7:1479-1485.
- Xiong YL, Mullins OE, Stika JF, Chen J, Blanchard SP and Moody WG (2007) Tenderness and oxidative stability of post-mortem muscle from mature cows of various ages. *Meat Science* 77:105-113.
- Young J, Karlsson A, Henckel P (2004) Water-holding capacity in chicken breast muscle is enhanced by pyruvate and reduced by creatine supplements. *Poultry Science* 83: 400-405.