



# Analysis of scenarios to reduce the probability of acquiring hemolytic uremic syndrome associated with beef consumption

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## Abstract

The objective of this study was to develop a quantitative microbial risk assessment (QMRA) model to evaluate potential risk mitigation strategies to reduce the probability of acquiring hemolytic uremic syndrome (HUS) associated with beef consumption in Argentina. Five scenarios were simulated to evaluate the effect of interventions on the probability of acquiring HUS from Shiga toxin-producing *Escherichia coli* (STEC)-contaminated ground beef and commercial hamburger consumption. These control strategies were chosen based on previous results of the sensitivity analysis of a baseline QMRA model (Brusa et al., 2020). The application of improvement actions in abattoirs not applying Hazard Analysis and Critical Control Points (HACCP) for STEC would result 7.6 times lower in the probability that consumers acquired HUS from ground beef consumption, while the implementation of improvements in butcher shops would lead to a smaller reduction. In abattoirs applying HACCP for STEC, the risk of acquiring HUS from commercial hamburger consumption was significantly reduced. Treatment with 2% lactic acid, hot water and irradiation reduced 4.5, 3.5 and 93.1 times the risk of HUS, respectively. The most efficient interventions, in terms of case reduction, being those that are applied in the initial stages of the meat chain.

## Keywords

hemolytic uremic syndrome, shiga toxin-producing *escherichia coli*, quantitative microbiological risk assessment, beef, scenario analysis

Date received: 30 April 2021; accepted 25 August 2021

## 1. INTRODUCTION

Shiga toxin-producing *Escherichia coli* (STEC) are important pathogens that cause diarrhea, hemorrhagic colitis and hemolytic uremic syndrome (HUS) in severe cases (Byrne et al., 2020). According to the World Health Organization Foodborne Disease Study, STEC infections produce 1.2 million foodborne median cases and 128 deaths annually worldwide (Kirk et al., 2015).

The consumption of contaminated meat, dairy products, vegetables, fruit and water as well as the contact with

animals and person-to-person transmission have all been associated with STEC infections (Costa et al., 2020a).

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The animal gastrointestinal tract, the environment and handlers' hands are common sources of meat-borne pathogen spread, particularly if there are poor sanitary conditions (Ncoko et al., 2020). This highlights the importance of implementing Good Hygiene Practices (GHP), Good Manufacturing Practices (GMP) and Hazard Analysis and Critical Control Points (HACCP) recognizing STEC as a hazard (HACCP-STECC) in food processing establishments (Casas et al., 2019; Guyon et al., 2001; Loiko et al., 2016).

The beef industry uses various risk mitigation strategies to reduce the number of pathogens in beef and beef products (Kocharunchitt et al., 2019). There is a wide range of antimicrobial interventions currently available for carcass processing, such as hot water washes, acid-base treatments (Zheng et al., 2019) and irradiation (Kawasaki et al., 2019), among others (Signorini et al., 2018).

Quantitative microbial risk assessment (QMRA) models related to STEC in beef, ground beef and hamburgers have been developed in different countries (Delignette-Muller and Cornu, 2008; Duffy et al., 2006; Ebel et al., 2004; Signorini and Tarabla, 2009). They contribute to evaluating different scenarios for risk management strategies (pre-slaughter screening, storage temperature control, herd vaccination, consumer information program) aimed at reducing the risk of STEC infection along the beef supply chain through sensitivity analysis (Signorini and Tarabla, 2010).

The objective of this study was to develop a QMRA model to evaluate potential risk mitigation strategies to reduce the probability of acquiring HUS associated with beef consumption in Argentina. The results of this study can help policy and research decision-makers to target interventions and resources to reduce the public health burden of STEC in beef products.

## 2. MATERIALS AND METHODS

### 2.1. QMRA Baseline model

The baseline model used in this study has been previously described in Brusa et al. (2020). Basically, a probabilistic risk assessment model was developed to characterize STEC prevalence (%) and contamination levels (log CFU/g) through five production modules of the beef supply chain: cattle primary production, cattle transport, processing and storage in the abattoir, retail and home preparation, and consumption (Figure 1).

Two types of beef abattoirs in Argentina were modeled based on the implementation of the HACCP system: abattoirs that apply HACCP and define STEC as a hazard (HACCP-STECC) and abattoirs with no HACCP plans or HACCP plans that do not define STEC as a hazard (not applying HACCP-STECC) (Brusa et al., 2020). The baseline model also included three beef products (ground beef [any food product containing ground meat except beef burgers], commercial hamburgers and intact beef cuts) and two

production scenarios (whole process carried out in the HACCP-STECC abattoir to produce the three beef products, and beef transported to the butcher shop for additional processing to be sold as beef cut or ground beef). Beef consumption habits in Argentina (handling, consumption and cooking preferences) from the baseline model (Brusa et al., 2020) were also taken into consideration in the current model.

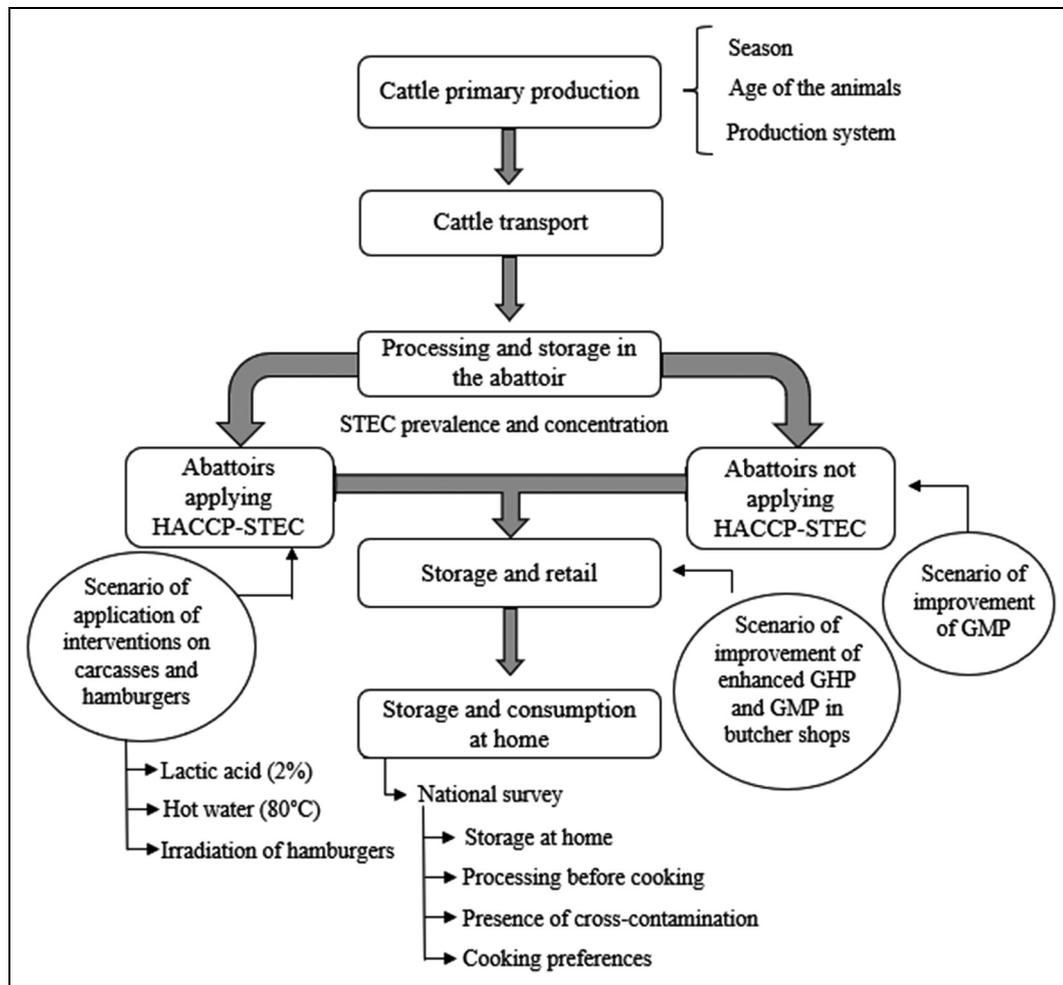
### 2.2. Risk Mitigation strategies

Strategies for STEC control were identified based on the results of the sensitivity analysis performed in the baseline model (Brusa et al., 2020). Accordingly, the food matrices included in the risk mitigation scenario analysis were ground beef and commercial hamburgers. Beef cuts were not included because the probability of clinical cases of HUS was not associated with this food matrix. The baseline model parameters and the risk mitigation scenarios are shown in Table 1.

#### 2.2.1. Ground Beef

*2.2.1.1. Improvement Of GMPs in abattoirs not applying HACCP-STECC.* Data from samplings carried out in abattoirs from Argentina by Costa et al. (2020b) were used to model the effect of the improvement actions in abattoirs not applying HACCP-STECC on the probability of acquiring HUS from ground beef consumption. In this study, STEC prevalence in carcasses was analyzed and the practices, facilities and equipment of the abattoirs were evaluated. Subsequently, improvements (building, processes, systems for water purification and training) were made to the abattoirs according to the initial diagnosis. After a period of adoption of these changes, the estimation of STEC prevalence in carcasses was repeated and the impact generated by the improvements was evaluated. In the scenario model, the rate of change in STEC prevalence per slaughter in abattoirs not applying HACCP-STECC was modified due to the prevalence obtained after the adoption of improvements.

*2.2.1.2. Improvement Of enhanced GHP and GMP in butcher shops.* The effect of improving GHPs and GMPs in butcher shops on HUS probability from ground beef consumption was modeled using data from a STEC prevalence study in Argentine butcher shops by Leotta et al. (2016). The mentioned study collected ground beef samples for the presence of STEC and proposed improvements on GHP and GMP based on an initial survey conducted on beef handling practices and the type of facilities and equipment used. A new prevalence study was conducted in ground beef to quantify the reduction of STEC prevalence and the level of success in the adoption of the proposed measures. The net effect generated (% reduction in cross-contamination rates and STEC prevalence in ground beef)



**Figure 1.** Modelo de línea de base y escenarios analizados.

by the application of enhanced GHP and GMP (hands washing and more frequent cleaning and disinfection of tables and utensils) was introduced in the baseline model scenario (Table 1).

### 2.2.2. Commercial Hamburgers

**2.2.2.1. Application Of lactic acid or hot water carcass wash.** The effect of carcass wash with 2% lactic acid or hot water at 80 °C using automated systems in abattoirs applying HACCP-STECC on HUS probability from hamburger consumption was modeled using data reported by Signorini et al. (2018) in Argentina. The net effect of both treatments separately on STECC prevalence (6.1 and 38.0% reduction, respectively) and generic *Escherichia coli* concentration (0.8 and 0.6 log reduction, respectively) on carcasses was modeled and compared with the baseline model (Table 1).

**2.2.2.2. Irradiation.** The Effect of irradiation on HUS probability from hamburger consumption was modeled using data reported by Cap et al. (2020). These authors

showed a net STECC >5 log CFU/g reduction (7 log CFU/g before and < 2 CFU/g after the intervention) with the application of 0.23 kGy. The scenario model considered that hamburgers positive for STECC before the intervention and negative after its application could still retain some microbial load, although STECC was not detected by current microbiological techniques (limit of detection, 2 log CFU/g, false negatives). The residual load of STECC in negative irradiated commercial hamburgers was estimated between 0.0003 CFU/g of hamburger (or the equivalent to 3 CFU/10 kg of hamburgers) and 0.00075 CFU/g of hamburger.

### 2.3. Scenario Analysis

The effect of the different risk mitigation scenarios was analysed in Microsoft Excel 2016 with the add-on package @Risk (version 7.5, Palisade Corporation, New York, USA). A Monte Carlo simulation with Latin Hypercube Sampling was used to assess all potential scenarios. Each simulation performed 5000 iterations of the model, which

**Table 1.** Baseline and scenario model parameters and risk mitigation scenarios.

Variable	Baseline model	Scenario model	References
<b>Improvement of GMP in abattoirs not applying HACCP-STECC scenario</b>			
Rate of change in STEC prevalence per slaughter in abattoirs not applying HACCP-STECC ( $\Pi_{(f-noH)}$ )	$\frac{\sim BETA(217+1; 401-217+1)}{PTr}$	$\frac{\sim BETA(42+1; 152-42+1)}{PTr}$	Costa et al. (2020b)
<b>Improvement of enhanced GHP and GMP in the butcher shop scenario</b>			
Probability of butchers washing their hands ( $P_{(LM)}$ )	$\sim Beta(1 + 1; 86 - 1 + 1)$	$\sim Beta(51 + 1; 86 - 51 + 1)$	Leotta et al. (2016)
Probability of butchers washing tables and counters ( $P_{(LT)}$ )	$\sim Beta(19 + 1; 86 - 19 + 1)$	$\sim Beta(53 + 1; 86 - 53 + 1)$	
Rate of change in STEC prevalence from meat cuts to ground meat ( $\Pi_{(cc-m)}$ )	$\frac{\sim BETA(239+1; 667-239+1)}{\sim BETA(8+1; 66-8+1)}$	$\frac{\sim BETA(33+1; 86-33+1)}{\sim BETA(8+1; 66-8+1)}$	
<b>Automated application of 2% lactic acid on carcasses in HACCP-STECC abattoirs scenario</b>			
Effect of the reduction in STEC prevalence by treating carcasses with 2% lactic acid ( $Red_{(AL2\%)}$ )		$\frac{\sim Beta(10+1; 150-10+1)}{\sim Beta(34+1; 150-34+1)}$	Signorini et al. (2018)
STECC prevalence on carcasses after treatment with 2% lactic acid ( $P_{(AL2\%)}$ )	$\frac{(P(Tr) \times TT(f-H))}{((1-P(Tr)) + (P(Tr) \times TT(f-H)))}$	$\frac{(P(mr-H) \times Red(AL2\%))}{((1-P(mr-H)) + (P(mr-H) \times Red(AL2\%)))}$	
Reduction in STECC concentration on carcasses after treatment with 2% lactic acid ( $Red_{(CAL2\%)}$ )		$\sim PERT(0, 51; 0, 8; 1, 08)$	
STECC concentration on carcasses after treatment with 2% lactic acid ( $C_{(mr-AL2\%)}$ )	$C(f-H) \sim Normal(2, 367; 0, 89(Truncado(0, 18; 5, 06)))$	$C(f-H) - Red(CAL2\%)$	
<b>Automated application of hot water at 80 °C on carcasses in HACCP-STECC abattoirs scenario</b>			
Effect of the reduction in		$\frac{\sim Beta(57+1; 150-57+1)}{\sim Beta(98+1; 150-98+1)}$	Signorini et al. (2018)

(continued)

Table 1. Continued.

Variable	Baseline model	Scenario model	References
STEC prevalence by treating carcasses with hot water ( $Red_{(AC)}$ )			
STEC prevalence on carcasses after hot water treatment ( $P_{(AC)}$ )	Reduction in STEC concentration on carcasses after hot water treatment ( $Red_{(AC)}$ ) $\sim PERT(0, 427; 0, 699; 0, 97)$	$\frac{(P(mr-H) \times Red(AC))}{((1-P(mr-H)) + (P(mr-H) \times Red(AC)))}$	
STEC concentration on carcasses after hot water treatment ( $C_{(mr-AC)}$ )	$C(f-H) \sim Normal(2, 367; 0, 89(Truncado(0, 18; 5, 06)))$	$C(f-H) - Red(AC)$	
<b>Commercial hamburger irradiation scenario (dose = 0.23 kGy)</b>			
Effect of STEC prevalence reduction by treating trimmings with irradiation ( $Red_{(I)}$ )		$\frac{\sim Beta(0+1; 90-0+1)}{\sim Beta(30+1; 30-30+1)}$	Cap et al. (2020)
STEC prevalence on trimmings after irradiation treatment ( $P_{(I)}$ )	$\frac{(P(mrH) \times OR(rec))}{((1-P(mrH)) + (P(mrH) \times OR(rec)))}$	$\frac{(P(rec) \times Red(I))}{((1-P(rec)) + (P(rec) \times Red(I)))}$	

allowed to achieve an adequate level of convergence (<1%). Model outputs were estimated as risk per serving of contaminated beef and population risk (median number of cases and 95% confidence intervals).

### 3. RESULTS

#### 3.1. Ground Beef

**3.1.1. Application Of improvement actions in abattoirs not applying HACCP-STECC.** The proposed improvements in the hygiene practices of the abattoirs not applying HACCP-STECC would result 7.6 times lower in the probability that consumers acquired HUS from ground beef consumption compared to the baseline model. That is, HUS cases in Argentina would reduce from 28 to 6 (Table 2). Although there is no precise information on the proportion of beef that is sold in the country by these establishments, in this risk assessment we considered that most of it came from abattoirs not applying HACCP-STECC (62.0%). This explains the impact that an improvement in processing conditions would have on the public health of consumers.

**3.1.2. Application Of GHP and GMP in butcher shops.** The implementation of improvements in butcher shops would lead to a lower reduction in the probability of acquiring HUS from the consumption of ground beef as compared with abattoirs not applying HACCP-STECC. In general, the risk of HUS as a result of consuming ground beef from butcher shops without GMP or GHP is 2.8 times higher (OR) compared with butcher shops that adopt GMP and GHP. That is, HUS cases in Argentina would reduce from 28 to 22 (Table 2).

#### 3.2. Commercial Hamburgers

**3.2.1. Application Of lactic acid or hot water carcass wash.** The application of interventions in the slaughter line of abattoirs applying HACCP-STECC significantly reduced the risk of acquiring HUS from commercial hamburger consumption. Treatment with 2% lactic acid and hot water implied 4.5 and 3.5 times lower risk of developing HUS, respectively, compared with the baseline scenario, which did not consider the application of any intervention. That is, HUS cases from consumption of commercial hamburgers in Argentina would reduce from 4 to 1 (Table 3).

**Table 2.** Results of the scenarios simulated to evaluate the effect of interventions on the probability of acquiring HUS from STEC-contaminated ground beef consumption in Argentina.

	Baseline model	Improvement of GMPs in abattoirs not applying HACCP-STECC	Improvement of enhanced GHP and GMP in butcher shops
<b>P<sub>(HUS)</sub> &lt;15 years from ground beef consumption</b>	$5.4 \times 10^{-8}$ ( $3.5 \times 10^{-10}$ - $3.9 \times 10^{-4}$ )	$7.4 \times 10^{-9}$ ( $1.3 \times 10^{-10}$ - $3.7 \times 10^{-5}$ )	$2.0 \times 10^{-8}$ ( $1.9 \times 10^{-10}$ - $3.0 \times 10^{-4}$ )
<b>Number of HUS cases &lt;15 years from ground beef consumption of</b>	28	6	22
<b>OR models of ground beef consumption</b>	Reference	7.6	2.8

3.2.2. *Irradiation Of hamburgers.* When the application of low doses of irradiation in commercial hamburgers was simulated, the probability of acquiring HUS from hamburger consumption was also greatly reduced (93.1 times). In other words, HUS cases reduced from 4 to 0 (Table 3).

#### 4. DISCUSSION

In this study, we evaluated the effectiveness of improvement actions and interventions in reducing the probability of acquiring HUS from STEC-contaminated ground beef and commercial hamburgers in Argentina, using a stochastic simulation model. Simulation models aim to generate scenarios which can serve as a basis for determining the most effective risk management measures to reduce the risk of STEC infection. The scenarios modeled in this work were chosen from the results of a sensitivity analysis applied to a baseline model reported earlier (Brusa et al., 2020).

Previous scenario analyses (Delignette-Muller and Cornu, 2008; Kiermeier et al., 2015) have reached similar conclusions. Our results coincide with other studies reporting that the identification of GHP and GMP in abattoirs and

STEC inactivation on carcasses are important control measures (Duffy et al., 2006), while it is difficult to obtain significant changes in butcher shops because they receive raw material that is already contaminated (Nauta et al., 2001).

The analysis of scenarios carried out in the present work showed that the impact of corrective actions on abattoirs without HACCP-STECC from Argentina would reduce HUS cases from ground beef consumption from 28 to 6. The lack of GMP, GHP and HACCP application has been associated with increased risk of contamination in abattoirs from our country (Brusa et al., 2017; Costa et al., 2020b) and different parts of the world (Bersisa et al., 2019; Casas et al., 2019; Douglas et al., 2013; Festus Jaja et al., 2018), with the consequent greater risk of contamination of the final product. This shows the need to work throughout the beef agribusiness chain to implement actions aimed at promoting a single sanitary standard based on the implementation of HACCP and considering STEC as a biological hazard (Costa et al., 2020a).

On the other hand, the scenario analysis of GHP and GMP application in butcher shops also showed a reduction, even though smaller, in HUS cases from 28 to 22 cases.

**Table 3.** Results of the scenarios simulated to evaluate the effect of interventions on the probability of acquiring HUS from STEC-contaminated hamburgers in Argentina.

	Baseline model	Automated application of 2% lactic acid on carcasses in HACCP-STECC abattoirs	Automated application of hot water at 80 °C on carcasses in HACCP-STECC abattoirs	Irradiation of hamburgers
<b>P<sub>(HUS)</sub> &lt;15 years from hamburger consumption</b>	$3.5 \times 10^{-8}$ ( $3.0 \times 10^{-10}$ - $2.0 \times 10^{-4}$ )	$7.8 \times 10^{-9}$ ( $2.0 \times 10^{-10}$ - $1.1 \times 10^{-4}$ )	$1.0 \times 10^{-8}$ ( $2.4 \times 10^{-10}$ - $1.8 \times 10^{-5}$ )	$3.8 \times 10^{-10}$ ( $5.3 \times 10^{-12}$ - $2.8 \times 10^{-6}$ )
<b>Number of HUS cases &lt;15 years from hamburger consumption</b>	4	1	1	0
<b>OR models of hamburger consumption</b>	Reference	4.5	3.5	93.1

Such lower reduction may be related to the fact that butcher shops are supplied by abattoirs applying or not HACCP-STECC, with unknown relative proportion (Brusa et al., 2020; Costa et al., 2020b). In other words, contamination could come from raw material. Likewise, as proposed in this scenario, several authors have identified problems in butcher hands washing and incorrect hygiene practices with utensils (Khanal and Poudel, 2017; Oliveira Vidal et al., 2020; Santos et al., 2017), evidencing the need to reinforce and consolidate the implementation of hygienic actions to improve and verify processes in all butcher shops (Barril et al., 2019).

The application of single or multiple intervention on carcasses and beef cuts contributes to reducing the prevalence of STECC in abattoirs with HACCP-STECC. The effectiveness of interventions at different points in the beef industrialization process has been demonstrated in numerous scientific works (Algino et al., 2007; Bosilevac et al., 2006; Geornaras et al., 2012; Kalchayanand et al., 2012; Kalchayanand et al., 2015). A study conducted in beef processing plants in the USA showed that the prevalence of STECC decreased from 58.3% to 8.3% in carcasses previously treated with different antimicrobial intervention strategies, including aspiration and steam, washing with hot water, organic acid washing and steam pasteurization (Arthur et al., 2002). The application of organic acids has also proved to be useful in reducing the prevalence of STECC in beef (Duffy and McCabe, 2014; Mohan and Pohlman, 2015).

In Argentina, different procedures to reduce the prevalence of STECC on carcasses through physical and chemical decontamination (Signorini et al., 2018) and on beef cuts and trimmings under controlled conditions (Cap et al., 2019) have been evaluated. In agreement with other authors (Algino et al., 2007; Bosilevac et al., 2006; Greig et al., 2012; Kanankege et al., 2017), the interventions showing the best results were automated application of 2% lactic acid and 3.5% lactic acid at 55°C, and hot water at 85 °C for 4 s (Signorini et al., 2018). In the present model, treatment with organic acids and hot water improved the quality of the meat produced in abattoirs, which would result in an improvement in public health. Among physical interventions, gamma irradiation is a well-known, safe and effective method for controlling microorganisms (Cap et al., 2020; Sommers et al., 2015; Xavier et al., 2014). In the scenario analysis of ground beef and hamburgers, it was demonstrated that the irradiation treatment would reduce 93.1 times the risk of HUS compared with the baseline scenario and considering the different population groups. This intervention would have the greatest impact on the probability of suffering from HUS. However, negative consumer opinion regarding food irradiation hinders its widespread adoption (Stratakos and Grant, 2018).

These results show that the most efficient interventions, in terms of case reduction, are those applied in the initial

stages of the meat chain; in the final stages of the chain, any attempt to improve the quality of the meat is limited and it only maintains the initial risk but does not reduce it. Thus, this analysis of scenario provides scientific evidence for decision-making at the level of health authorities, to reduce the casuistry of HUS in Argentina.

## ACKNOWLEDGEMENTS

The authors thank A. Di Maggio for manuscript correction and editing.

## DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

## FUNDING

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This study was supported by research grants from the Institute for the Promotion of Argentine Beef, IPCVA ([www.ipcva.com.ar](http://www.ipcva.com.ar)).

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## SUPPLEMENTAL MATERIAL

Supplemental material for this article is available online.

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