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Use of different cold water spray protocols on bovine carcasses during cooling and its effects on meat quality

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Abstract. This study was designed to evaluate the cold-water spraying (2 °C) on bovine carcasses during cooling, and its effects on meat quality. In protocols I, II, and III, 60-second spraying cycles were performed 10 min apart, with every cycle lasting 13 h, 15 h, and 17 h, respectively. In protocol IV, a software-controlled refrigeration unit was used, determining the interval and duration of the spray cycles. Data evaluated by counting colony forming units before the cooling period and after 24 h of carcass cooling were carcass features, such as body weight at slaughter, warm and cold carcass weight, weight losses on cooling, pH, temperature, and microbiological quality. Meat samples were collected to evaluate the *Musculus longissimus thoracis* for color, fat thickness, losses on cooking, loss of exudation, and shearing force, shortly after boning, and after 14 days of maturation. The protocols were effective in controlling the weight loss during cooling. The 17-hour protocol presented weight loss similar to that of the software-controlled. No significant difference was observed for the shearing force (kgF) evaluated in the same maturation period. The protocols tested did not create a favorable environment for the growth of psychrotrophic and enterobacteria in the evaluated carcasses. The use of the spray contributes to the reduction of mesophiles after 24 h of cooling. Differences between the results of the protocols for losses on exudation, losses on cooking, and color were observed, and the software-controlled protocol presented the highest loss values due to exudation, cooking, and luminosity.
Keywords: beef, cycles, losses on cooling, microorganisms

Introduction

In the refrigeration industries, due to the enzymatic and high-water activities, after slaughtering, the cattle carcasses require storage and conservation procedures. Among the methods adopted for this purpose, refrigeration is the most widely used. This method aims to control the proliferation of microorganisms by reducing the temperature, inhibiting the development of pathogenic and deteriorating microorganisms, and delaying enzymatic activities that contribute to physical and sensory alterations (Stevenson, Merkel and Lee, 1978; Lawrie, 2005).

The refrigeration of bovine carcasses consists of storing them inside cooling chambers with a temperature ranging from 0 °C to 4 °C for a 24-hour average period. This conventional cooling process through forced ventilation causes weight loss, technically referred to as a "Cold Break". According to Smith & Carpenter (1973), the weight loss occurring during the cooling process is the result of evaporation, water drip washing, and exudation of carcass components. It is estimated that these weight losses occurring during conventional cooling can reach 20 g/kg of the warm carcass weight (James, 1996). A survey carried out in Brazil showed weight losses ranging from 4.0 g/kg to 22.3 g/kg (Sampaio, 2016).

Due to the major economic impact caused by weight losses during cooling, refrigeration industries have looked for alternatives to mitigate this effect. One of the potential alternative techniques is the spraying of cold water on the surface of the carcass during cooling. This technique helps reduce the temperature of the half-carcasses during cooling processes and prevents microbiological quality, softness, and meat pH impairment (Jones & Robertson, 1988; Greer et al., 1990; Greer & Jones, 1997).

Moreover, to eliminate the risk of weight gain during spraying, a spray pattern can be used in which the refrigeration unit is controlled by software that records the weight of the carcass in real time and determines the interval and duration of the spraying cycles (Anderson et al. 1993). Additionally, this helps minimize other undesirable effects of prolonged spray cycles, such as color change, formation of pockets, washed aspect, and increased exudation of vacuum-packed cuts.

Thus, the aim of this study was to evaluate different protocols to determine which one results in the smallest weight losses during the cooling of bovine carcasses, without changes in the qualitative and microbiological parameters of the meat.

Materials and Methods

The study was carried out in the northern region of the Mato Grosso State, Brazil, latitude 11°5151″ S and longitude 55°3008″ O.

Four spraying protocols were carried out. In protocols I, II, and III, 60-second spraying cycles were performed 10 min apart, with every cycle lasting 13 h, 15 h, and 17 h, respectively. In protocol IV, a software-controlled refrigeration unit was used, recording the carcass weight in real time and determining the interval and duration of the spray cycles. In all treatments, the temperature of the water sprayed on the carcasses was 2 °C.

One hundred and twenty animals, namely 30 bovine carcasses of Nellore females, aged 18 to 24 months according to the evaluation of the dental chronology (two permanent incisor teeth), fat thickness ranging from 3 mm to 6 mm, and carcass weight between 180 kg and 210 kg were used.

The animals were slaughtered in a state-inspected slaughterhouse, according to the rules recommended by the Industrial and Sanitary Inspection Regulations for Animal Products (RIISPOA) (Brazil, 2017). The animals remained in a rest, fasting, and water diet for a period ranging from 12 h to 24 h before being taken to the slaughter room. At the time of slaughter, all animals were desensitized with a penetrating captive dart pistol (USSS-1, Jarvis-USA model), and bleeding procedures were immediately performed with sagittal skin opening in the ventral midline of the neck and section of the large vessels of the thorax region, and sagittal cut of the jugular veins and carotid arteries. After bleeding the animals, a low voltage electrical stimulation was applied in the lateral region through a stimulator (BV 80, Jarvis-USA model).

After weighing, the carcasses were washed and taken to the carcass cooling chamber, where they underwent a conventional cooling method, with temperatures ranging from 0 °C to 4 °C and an air speed of 0.5 m/s for a period of 24 h.

In the hindquarters, the area between the 12th and 13th ribs was demarcated, the selected area was boned, and samples approximately 2.5 cm thick were taken from the *Musculus longissimus thoracis*. Two samples were collected from each half carcass during maturation periods of 0 to 14 days. The samples were individually vacuum-packed using a heat-shrinkable film package (model 22x40, Cryo-Vac®, Brazil) and duly identified. They were then separated in cardboard boxes according to the maturation time, and taken to the Technology Maturation Chamber at temperatures ranging from 0 °C to 2 °C. The cartons containing the samples were placed on metal shelves to avoid stacking. After the respective maturation periods, the samples were removed from the chamber and subjected to laboratory analysis.

The qualitative aspects of the carcass were evaluated by calculating the weight loss on cooling (g/kg), and by measuring the pH and temperature of the carcasses subjected to the 04 procedure (four cold water spray protocols during cooling).

The animals were individually weighed immediately prior to slaughter, after fasting, rest, and watering in the pens, with the aid of a trunk containment scale (model WT 3000-I, Weigthtech®, Brazil) installed in front of the stall, obtaining their body weight at slaughter (BWS). After slaughter, half of the carcasses were weighed with a railing scale

(model 9091, Toledo®, Brazil), obtaining the warm carcass weight (WCW). After the 24 h cooling period, the half carcasses were weighed with a railing scale (model 9091, Toledo®, Brazil), obtaining the cold carcass weight (CCW).

The percentage of losses on cooling (LOC) was obtained by calculating

$$1.\ LOC = \left(\frac{WCW - CCW}{WCW}\right) * 100$$

WCW: Warm carcass weight;
CCW: Cold carcass weight.

The pH was measured in the *Musculus. longissimus thoracis* at the 12th to 13th ribs, using a portable pH meter manufactured by Linelab®, model pH Classic, with a 7 cm needle, and 2 cm from the tip of the knife. Measurements were performed at time 0 h, 2 h, 6 h, 10 h, 14 h, and 24 h of cooling. The pH meter was calibrated before the data collection began.

The temperature was measured in the internal muscular region of the topside (*Musculus obturatorius internus, Musculus semimembranosus*) with a digital "spit-type" thermometer manufactured by Akso® model AK 02, through the filled foramen. Measurements were performed at time 0 h, 2 h, 6 h, 10 h, 14 h, and 24 h of cooling. The thermometer was calibrated before collecting the information.

For the microbiological assessment, the APHA (2001) methodology was used to count mesophilic, psychrotrophic, and enterobacterial bacteria.

Samples were collected on the muscle surface of three carcasses per treatment immediately after washing (time 0 h), and from the same three carcasses after 24 h of cooling (time 24 h). These three carcasses were randomly selected from the 10 carcasses used for each treatment. Each carcass sample was collected in duplicate. The samples were obtained with the aid of a sterile hydrophilic cotton-tipped swab applied to the topside, loin, and forequarter in 100 cm² areas at each point, using a stainless-steel jig (APHA, 2001).

After the application at the three studied points, the swab rod was introduced into a vial containing 10 mL of 0.1% peptone saline solution (10° tube). These containers were transported in Styrofoam boxes immediately after collection for preparation at the Food Microbiology Laboratory. The collection tube (10°) was homogenized and 1 ml of the inoculation solution was removed into another vial containing 9 mL of 0.1% peptone saline solution (tube 10^{-1}). Sample 10^{-1} was homogenized, and 1 mL of the inoculation solution was withdrawn into another vial containing 9 mL of 0.1% peptone saline solution (tube 10^{-2}).

Counts were performed by the surface plating method with a standard counting agar (SCA), and subsequently incubated. For the counting of mesophilic bacteria, the dilution plates (10^0, 10^{-1}, and 10^{-2}) were transferred into a bacteriological incubator at 35 °C ± 1 °C for 48 h. For the counting of psychrotrophic bacteria, the dilution plates (10^0, 10^{-1}, and 10^{-2}) were transferred into bacteriological incubators at 7 °C ± 2 °C for 10 days. The enterobacterial counts were performed using the deep plating method with Violet Red Bile Glucose Agar (VRBG), and then inverted and incubated in a stove at 30 °C ± 1 °C for 24 h.

In order to obtain the number of mesophilic, psychrotrophic, and enterobacterial bacteria, the number of colony-forming units in the plates was counted and multiplied by the corresponding dilution per cm² of the carcass (APHA, 2001).

In order to assess the physicochemical characteristics of meat, the color and fat thickness of the vacuum-packed meat samples were checked within 0 days, and the losses on cooking, exudation losses, and shearing force of the vacuum-packed meat samples were submitted to the four protocols of cold-water sampling at time 0 and 14 days during the proposed cooling process.

To verify the staining parameters, a colorimeter (CR-410 model, Minolta®, Brazil) was used, in which the evaluated parameters were L*, a*, and b* hues of the CIElab system, where L* represents luminosity, and a* and b* are the red and yellow color intensities, respectively. Three measurements were taken at different points from each sample, and their means were considered.

To determine the subcutaneous fat thickness, the measurement was performed with the aid of a digital caliper (Absolute Model, Mitutoyo®, Brazil) of meat's covering fat sample collected between the 12th and 13th ribs.

To verify losses on leakage, packed samples were weighed and removed from the packaging. The packages were emptied, dried, and weighed separately. Losses on exudation were calculated by the ratio of the weight difference of the packed samples and packages.

For losses on cooking, the samples were weighed and a "skewer-type" digital thermometer (AK 02 model, Akso®, Brazil) was inserted into their geometric center. The samples were then cooked on a grill plate (G-01 model, Mondial®, Brazil) until they reached 40 °C; they were then turned and the same previous procedure was performed until they reached 71 °C, when they were removed. The samples were cooled at a natural temperature of 24 °C for 60 min, and the losses on cooking were subsequently measured, as proposed by Wheeler et al. (1995), where the loss of cooking was calculated by the ratio of the difference in initial and final weight.

The shearing force analysis was performed after the samples completed the cooking test. The samples were foil-packed and stored under refrigeration at 2 °C ± 2 °C for 24 h. After the refrigeration period, 10 meat cylinders with a 12 mm diameter were removed with a bench drill (FG13 model, Rexon®, Brazil) from each sample, to ensure the reliability and accuracy of the results. The cylinders were analyzed using a mechanical texture analyzer (TA XT-Plus Texture Analyzer 2i, Stable Micro System, UK) equipped with a Warner-Bratzler

accessory, 25 kg capacity, and 20 cm/min disconnector speed, according to the methodology proposed by Wheeler, Koohmaraie and Shackelford (1995), updated by Savell et al. (2017).

Statistical analysis

For the analysis of the effect of the protocols on weight loss during cooling, a completely randomized design (CRD) was considered, including the body weight at slaughter as covariate, and comparing the averages by the Tukey-Kramer test with 5% significance, as per the statistical model 2:

$$2.\ Yijk = \mu + Ti + Rj + \mu j\ eijk.$$

Where:
Yijk: corresponds to the ijk observation;
μ: corresponds to the general average;
Ti: corresponds to treatments (i = Protocols I, II, III, and IV);
Rj: cooling (j = before or after cooling);
Eijk: corresponds to the ijk observation-related error.

For the statistical analyses of quantified microbial parameters on carcass surfaces, a CRD was considered immediately after washing and 24 h after spray-cooling, comparing the averages by the Tukey-Kramer test with 5% significance, as per the statistical model 3 below:

$$Yijk = \mu + Ti + Rj + \mu j\ eijk.$$

Where:
Yijk: corresponds to the ijk observation;
μ: corresponds to the general average;
Ti: corresponds to treatments (i = Protocols I, II, III, and IV);
Rj: cooling (j = before or after cooling);
eijk corresponds to the ijk observation-related error.

For the statistical analyses of the qualitative parameters and physical-chemical features of the meat, a CRD was considered, comparing the averages by the Tukey-Kramer test with 5% significance, as per the statistical model 4 below:

$$Yijk = \mu + Ti + Mj + \mu j\ eijk.$$

Where:
Yijk: corresponds to the ijk observation;
μ: corresponds to the general average;
Ti: corresponds to treatments (i = Protocols I, II, III, and IV);
Mj: maturation (j = maturation: 0 to 14 days);
eijk corresponds to the ijk observation-related error.

Statistical procedures were performed using SAS (Statistical Analysis System, version 9.3.)

Ethical principles and good practices in experimentation

All animals used in this research were slaughtered according to the current regulations in accordance with the State Health Inspection Service under number: 011 SISE-MT.

Results and discussion

A treatment effect (P<0.05) was observed between protocols I and II for the losses on weight during cooling, but not between protocols III and IV, which showed no difference between them (P>0.05). Protocols III and IV had the lowest weight loss values during cooling (Table 1).

No difference in subcutaneous fat thickness (P>0.05) was observed between the carcasses submitted to the four spraying protocols tested. Furthermore, no protocol used in this survey showed carcass weight gain (P>0.05).

The average temperature and pH values of the carcasses subjected to protocols I, II, III, and IV were evaluated at 0 h, 2 h, 6 h, 10 h, 14 h, and 24 h, respectively, and are presented in Table 2. The temperature reduction of protocols I, II, and III was similar, and there was no treatment effect (P>0.05) in any of the evaluated cooling periods (Table 2). Additionally, the final temperature values after 24 h of cooling were not affected (P>0.05) by the different proposed protocols. In contrast, protocol IV showed a statistical difference (P<0.05) in temperature reduction at 2 h, 10 h, and 14 h of cooling, compared to the other protocols.

Protocols II and III presented similar values in the pH curve, with no difference (P>0.05) between treatments in all evaluated periods. Protocol I presented a statistical difference (P<0.05) at 24 h compared to protocols II and III. However, the values presented in these protocols showed no difference (P>0.05) compared to that verified in protocol IV (Figure 1).

The treatment effect (P >0.05) on shearing force (kgF) showed no difference between the protocols at both the 0 day and 14 days maturation time (Table 3). However, when the average shearing force at 0 day maturation time (6.04 kgF) was compared to the average 14 days maturation time (4.73 kgF), a treatment effect was observed (P<0.05).

The loss of cooking values of samples submitted to protocols I, II, and III at 0 day and 14 days maturation times were similar (Table 3). Protocol IV presented similar loss on cooking values to protocol I at 0 day maturation time, and similar values to protocols I and III at 14 days maturation; however, protocol IV showed a difference (P<0.05) when compared to protocols II and III at 0 day maturation time, and to protocol III at 14 days maturation time cycles. The average cooking loss value at the 0 day maturation was similar to that of the 14 days maturation time.

The loss of exudation results for the 0 day maturation time were similar (P>0.05) among the tested protocols. However, when losses on exudation at 14 days maturation time were evaluated, there was a difference (P<0.05) between protocols I and IV. When comparing the average losses on exudation at

0 day maturation time to the average losses at 14 days maturation time, a treatment effect (P<0.05) was observed.

The values of a* and b* hues were similar (P>0.05) for all treatments (Table 4). The L* values were similar (P>0.05) for protocols I, II, and III. Protocol IV presented similar values (P>0.05) to protocol I, but there was a difference (P<0.05) between this protocol, and protocols II and III. In this experiment, it was observed that the use of spray, regardless of the proposed protocol, created an aspect of washed and bleached color in the analyzed carcasses.

The CFU/cm² values of mesophiles were significantly different (P<0.05) among the protocols (Table 5).

The fat thickness in the carcass did not change through the protocols. This fact provides a level playing field in relation to possible weight losses, according to Fisher & Bauntun (1983). Lower fat coverage suggests a greater possibility of weight loss during cooling due to the lower amount of water in the tissues and/or the body barrier formed by the fat covering.

The results obtained in this experiment corroborate the results of the surveys carried out by several authors, which demonstrated significant reductions in weight loss during cooling when using a spraying system (Allen et al., 1987; Jones & Robertson, 1988; Greer et al., 1990; Hippe et al., 1991; Strydom & Buys, 1995; Greer & Jones, 1997; Mesquita et al., 2003; Prado & Felício, 2010).

The differences in weight loss reduction observed among protocols I, II, and III demonstrate, as described by Greer & Jones (1997), that the use of spray cycles with a longer total duration, hence, a larger number of cycles, is most effective in reducing weight loss. According to the same authors, there is a linear relationship between spraying time and weight loss reduction.

On the other hand, the spraying protocol I tested in this survey presented low efficiency in reducing weight loss during cooling compared to the results of the other protocols used in this survey. Spraying protocol II presented results similar to those of Allen et al. (1987), Jones & Robertson (1988), Greer et al. (1990), Mesquita et al. (2003), and Prado & Felício (2010) who found weight losses of 3.2 g/kg, 3.5 g/kg, 2.8 g/kg, 3.9 g/kg, and 3.4 g/kg, respectively. As far as protocol III is concerned, the results are close to those of Strydom & Buys (1995), who showed weight losses of 2.2 g/kg in the carcasses tested for the 17-hour spray cycle.

As suggested by Anderson et al. (1993), protocol IV was effective in reducing weight losses because the recorded values of weight losses were close to effect deletion. Protocol III presented reduction values close to those found in protocol IV, but it must be emphasized that, due to its prolonged spraying time, some carcasses tested in this protocol presented undesirable alterations such as wet aspect, as verified by Strydom & Buys (1995), and Greer & Jones (1997), or surface ice crystals. These changes were not observed in protocol IV, which presented normal-looking carcasses without the formation of surface ice crystals. This is probably due to the fact that, since the cycles are not performed with an established duration and interval, but controlled according to the weight reduction verified in real-time by a balance, the cycles are adjusted according to the need, with no formation of superficial accumulation of water (Anderson et al., 1993).

In our study, the protocols did not cause carcass weight gain, but different results were observed by Prado & Felício (2010), who noticed a 7.0 g/kg weight gain in the first slaughter and a reduction to 0 g/kg in the second, with a 2.8 g/kg average weight gain at slaughter between the two slaughters, when using the conventional 48-hour cooling method with 6-hour spraying. These are important results because, according to the legislation that regulates the use of spraying in Brazil, carcass weight gain due to the cooling technique is not allowed (Brazil, 2018).

Although the final results of the protocols used in this survey were similar to those of other surveys, it has to be emphasized that the programming of the spraying system (total spraying time, total number of cycles, cycle duration, and interval between cycles), carcass characteristics (weight, sexual condition, fat thickness), and carcass spacing were variable among all studies, and were different from those used in this study.

This confirms the difficulty in establishing a protocol suitable for all situations due to the numerous possibilities of cycle adjustments (Prado & Felício, 2010) and the important differences to be considered when deciding to use this technique, ranging from the physical structure of the carcass cooling chambers (number of evaporators, evaporator capacity, air speed) (Sampaio, 2016) to the spray system (nozzle spacing, nozzle water pressure, and total volume of sprayed water) (Prado et al., 2007).

The temperature reduction of the carcasses was similar in all protocols, similarly to the results verified by Strydom & Buys (1995), who did not notice differences in the temperature curve between different spraying protocols (10, 14, and 17 h), and by Hippe et al. (1991), who also found no differences in the temperature reduction of carcasses subjected to spraying protocols with different duration periods.

The temperature values observed after 14 h of cooling were above 13 °C, which indicates that cold temperatures did not shorten the muscle fibers. According to Hannula & Puolanne (2004), the ideal is that carcasses are not cooled below 12 °C in less than 15 h, that is, before the complete establishment of rigor mortis. Moreover, the temperature reduction that occurred in 2 h, 10 h, and 14 h of cooling in the IV protocol suggest that during these periods the automated system reduced the interval between spraying cycles for weight adjustments, influencing the lower temperature value found in this protocol. This hypothesis is supported by the statement made by Jones & Robertson (1988) that spraying increases

the cooling rate, reducing the temperature by 1 °C to 2 °C.

The changes in pH observed between the spray protocols have not been reported previously. Thus, the differences in values may have been influenced by other variables, such as stress suffered by the animals during transport, prolonged fasting, or pre-slaughter conditions that directly influence the pH after 24 h of cooling (Silva, Patarata and Martins, 1999; Maltin et al., 2003; Jeleníková, Pipek and Staruch, 2008).

The 24-hour pH values found in this experiment were similar to those found in other studies such as Prado & Felício (2010), who found pH values between 5.5 and 5.6 after 24 h of cooling. Pearce et al. (2011) also corroborated these results by reporting that, in normal situations, the evolution of pH in cattle develops slowly, starting with an initial muscle pH of around 7.0, reaching 6.8 to 6.4 after 5 h, and falling to a level between 5.9 to 5.5 after 24 h.

The shearing force has not changed, and the results are similar to those found by Jones & Robertson (1988), Greer & Jones (1997), and Prado & Felício (2010), who also did not observe any differences in shearing force results in samples obtained from carcasses subjected to different spraying protocols.

The differences observed in the average shear force during the maturation time of 0 days, when compared with the average maturation time of 14 days, is similar to that reported by Andrade et al. (2010), who observed an effect of bovine meat maturation time on shearing force reduction, so that reductions between 1 and 7 days, 7 and 14 days, and between 14 and 21 days ranged from 1.09, 0.21, and 0.56 kgF, respectively. The reduction in shearing force from the maturation period is caused by the action of endogenous meat proteases that are favored by keeping the vacuum-packed meat under refrigeration (temperature around 2 °C ± 2 °C) for a 7- to 28-day period after slaughter (Puga, Contreras and Turnbull, 1999).

Cooking losses at 0 days and 14 days did not change, a similar result was observed by Prado & Felício (2010), who found no difference in losses on cooking during 7- and 14-day maturation periods in samples obtained from cool sprayed carcasses. Strydom & Buys (1995) also found no differences in losses on cooking of carcass samples that underwent different spray cycle periods. However, King et al. (1993) observed that there was an increase in cooking losses, from 249.0 g/kg after one day of maturation to 262.0 g/kg after 14 days of maturation. Losses on exudation at 14 days of maturation between protocols I and IV may arise from lower weight losses of carcasses submitted to protocol IV during cooling, compared to the losses arising from protocol I, which could suggest the possibility of greater exudation over a longer storage period.

The results of the average losses in the exudation at the maturation time of 0 days in comparison with the losses in the 14 days were different. However, this result is similar to that found by Allen et al. (1987), who observed a difference in the losses of exudation of vacuum-packed ribs for a 15-day period when subjected to spray cooling and Prado et al. (2007), who verified an increase in losses on exudation of samples from carcasses undergoing cooling using the spraying system after 14 days of maturation. The averages verified were 8.1 g/kg at 0 day and 17.1 g/kg at 14 days maturation time. According to Prado et al. (2007), these results indicate the possibility that the reduction in weight loss during cooling with spraying may be lost over longer storage periods of the vacuum-packed meat.

In contrast, Strydom & Buys (1995) did not observe differences in losses on exudation from vacuum packed cuts between the 10 h, 14 h, and 17 h spraying protocols tested, and Greer et al. (1990) and Greer & Jones (1997) also found no differences in losses on exudation in the different spraying protocols tested.

The values of a* and b* hues did not change with the spraying protocols, and similar results were found by Jones & Robertson (1988), who found no effect on instrumental color evaluation (a* and b* hues) in meat samples from carcasses subjected to 4-day, 8-hours, and 12-hours spray cycles. The same authors verified that there was a bias towards variation in L* values in the carcass meat samples undergoing spraying, compared to the control group without spraying. However, Greer & Jones (1997) observed no spray system effect on any of the L*, a*, and b* variables, when comparing carcass samples that were sprayed during cooling at 4 h, 8 h, 12 h, and 16 h cycles, respectively.

The use of spray protocols caused an aspect of washed and bleached color in the carcasses, which was also described by Greer & Jones (1997) and Prado et al. (2007).

Differences in CFU/cm² values of mesophiles between protocols may be caused by operating variations during the slaughter operations (Gill, 2004). An effect was also observed among the 0- and 24-hour evaluated periods, and according to Sampaio (2013), this characteristic is related to the harmful/damaging effect of cooling the mesophiles populations. However, there was no cooling period effect causing a reduction of enterobacterial counts, which, according to Sampaio (2013), is due to the greater adjustment ability of these bacteria to cooling conditions.

The results observed in this survey indicate that, regardless of the protocol used, spraying did not favor microbiological growth in carcasses. These results are similar to those verified by Greer et al. (1990), Strydom & Buys (1995), and Greer & Jones (1997), who also verified that there was no effect of spraying on carcass microbiological quality.

Different results were observed by Hippe et al. (1991) who, in a survey carried out, found an increase in total counts of mesophilic aerobic and facultative anaerobic microorganisms in sprays treated with the spraying system. They also observed that the counts of aerobic psychrotrophic, facultative anaerobic psychrotrophic, and lactic bacteria tended

to be higher in carcasses that were spray-cooled compared to those cooled by conventional methods. However, according to Mesquita et al. (2003), the use of carcass spraying associated with good hygiene conditions before, during, and after slaughter contributes to low microorganism counts, and the use of spraying does not compromise the bacteriological quality of meat, instead providing better quality than that of non-sprayed carcasses. Although the use of spraying causes an increase in carcass surface moisture, which could create favorable conditions for microbiological growth, such an effect can be minimized by the temperature reduction caused by spraying (Greer & Dilts, 1988).

Table 1. Average values and standard error to carcass weights and losses during cooling

Parameters	Protocols			
	I	II	III	IV
Body weight on slaughter, kg	358.14	358.14	358.14	358.14
Warm carcass weight, kg	200.58±1.00	201.93±0.99	199.29±1.04	200.70±1.01
Cold carcass weight, kg	199.55±1.00	201.14±0.99	198.91±1.04	200.34±1.01
Weight loss during cooling, kg	1.03±0.17a	0.79±0.17b	0.38±0.17c	0.36 ±0.17c
Weight loss during cooling, g/Kg	5.12±0.80a	3.88 ±0.80b	1.89±0.80c	1.82 ±0.80c
Fat thickness, mm	4.93±0.21	4.96±0.21	5.08±0.21	4.56±0.21

* Means followed by the same lowercase letter, in line, do not differ each other (P> 0.05), according to the Tukey-Kramer test.

Table 2. Average values and standard error to carcass temperature and pH according to spraying protocols: I (13 hours). II (15 hours). III (17 hours) and IV (software controlled)

Time	Protocols			
	I	II	III	IV
	Temperature			
0	38.86±0.21	39.41±0.21	39.63±0.21	38.85±0.21
2	33.99±0.21ab	33.84±0.21ab	34.98±0.21a	32.50±0.21a
6	26.77±0.21	26.76±0.21	27.15±0.21	26.12±0.21
10	20.00±0.21a	19.66±0.21a	19.97±0.21a	18.38±0.26a
14	15.47±0.21a	14.85±0.21a	15.31±0.21a	13.52±0.26a
24	4.46±0.21	4.37±0.21	4.73±0.21	4.66±0.21
Time	pH			
0	6.75±0.13a	6.75±0.13a	6.72±0.13a	6.82±0.13b
2	6.58±0.13a	6.64±0.13ab	6.56 ±0.13ac	6.60±0.13abc
6	6.40±0.13a	6.37±0.13a	6.36±0.13a	6.50±0.13b
10	6.20±0.13a	6.23±0.13ab	6.24±0.13ac	6.30±0.16bc
14	6.00±0.13a	6.11±0.13b	6.11±0.13b	6.07±0.16ab
24	5.68±0.13a	5.57±0.13b	5.56±0.13b	5.72±0.13a

* Means followed by the same lowercase letter, in line, do not differ each other (P> 0.05), according to the Tukey-Kramer test.

Table 3. Average values and standard error to shearing force (kgF), losses on cooking (g/Kg) and exudation (g/Kg) undergoing 0- and 14-day maturation periods, according spraying protocols: I (13 hours), II (15 hours), III (17 hours) and IV (software controlled)

	Protocols				
	I	II	III	IV	
Maturation	Shearing force (kgF)				Average
0 days	5.98±0.20A	6.18±0.20A	6.10±0.21A	5.89±0.20A	6.04±0.10A
14 days	5.04±0.20B	4.92±0.20B	4.80±0.20B	4.19±0.20B	4.73±0.10B
Maturation	Losses on cooking (g/Kg)				Average
0 days	210.1±5.2ab	200.1±5.4a	200.7±4.9a	218.8±4.9b	207.4±2.5
14 days	204.5±4.9ab	201.1±5.1a	206.1±4.9ab	221.1±5.2b	208.2±2.5
Maturation	Losses on exudation (g/Kg)				Average
0 days	6.6±0.8A	6.5±0.8A	7.4±0.8A	5.4±0.8A	6.5±1.0A
14 days	15.4±0.8Bb	17.6±0.9Bab	1.56±0.8Bab	19.5±0.9Bb	17.0±1.0B

* Means followed by the same lowercase letter, in the same line do not differ each other (P> 0.05), according to the Tukey-Kramer test. Means with the same capital letter do not differ besides de maturation time, according to the Tukey-Kramer test.

Table 4. Average values and standard error to meat sample color evaluation according spraying protocols: I (13 hours), II (15 hours), III (17 hours) and IV (software-controlled)

Hues*	Protocols			
	I	II	III	IV
L*	38.21±0.45ab	37.84±0.45a	37.91±0.44a	39.74±0.44b
a*	22.35±0.34	22.73±0.35	21.68±0.34	22.01±0.34
b*	7.79±0.29	8.06±0.29	7.48±0.33	8.23±0.28

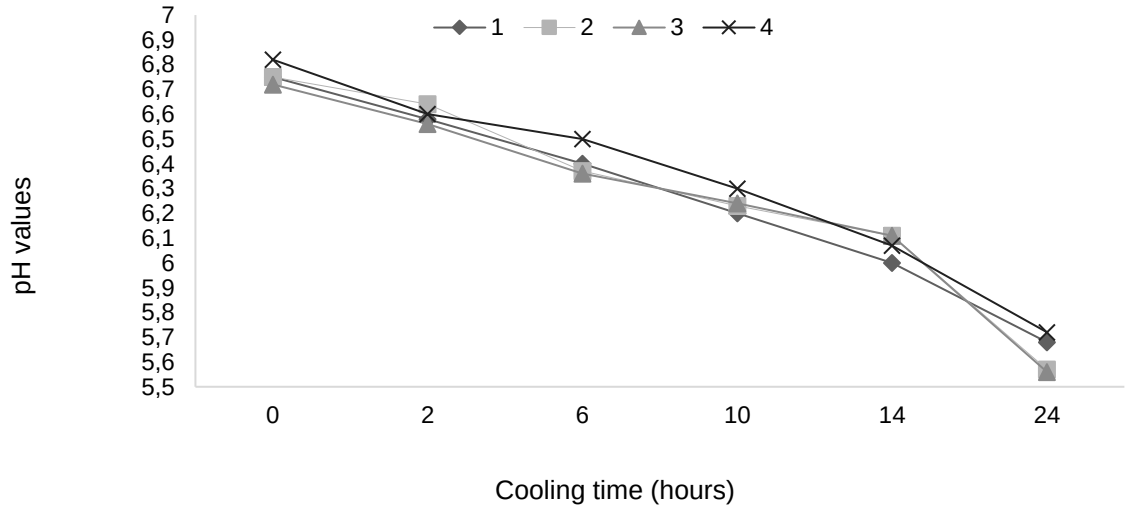
* Means followed by the same lowercase letter, in the same line, do not differ each other (P> 0.05), according to the Tukey-Kramer test.

Table 5. Average values and standard error of Colony Forming Units (CFU/cm²) over 0- and 24-hour cooling time according spray protocols: I (13 hours), II (15 hours), III (17 hours) and IV (software-controlled)

		Protocols				
		I	II	III	IV	Average
Enterobacteria	0	0.11±0.01	0.10±0.01	0.10±0.01	0.06±0.01	0.09±0.01
	24	0.10±0.01	0.09±0.01	0.09±0.01	0.06±0.01	0.08±0.01
Mesophiles	0	0.32±0.03Aa	0.24±0.03Aab	0.24±0.03Aab	0.16±0.03Ab	0.24±0.02A
	24	0.19±0.036Ba	0.11±0.03Bab	0.13±0.03Bab	0.05±0.03Bb	0.12±0.02B
Psychrotrophics	0	0.21±0.11	0.15±0.09	0.17±0.09	0.25±0.09	0.19±0.07
	24	0.17±0.11	0.12±0.09	0.30±0.09	0.25±0.09	0.20±0.06

*Averages followed by the same uppercase letter, in column; and lowercase letter in row, do not differ each other (P>0.05) by the Tukey-Kramer test.

Figure 1. pH values at 0, 2, 6, 10, 14, and 24 hours cooling time, according the protocols: I (13 hours), II (15 hours), III (17 hours) and IV (software-controlled)



Conclusion

Regardless of the protocol used, spraying was efficient in reducing heifer carcass weight losses during cooling. The 17-hour protocol showed weight loss reduction results similar to the software-controlled protocol results, indicating that it can be used instead. There was no influence of spraying protocols on the shearing force. The use of spray during cooling did not create a favorable environment for enterobacterial and psychrotrophic growth after 24 h of cooling. Regardless of the protocol used, spraying helped reduce the count of mesophilic colony-forming units after 24 h of cooling.

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