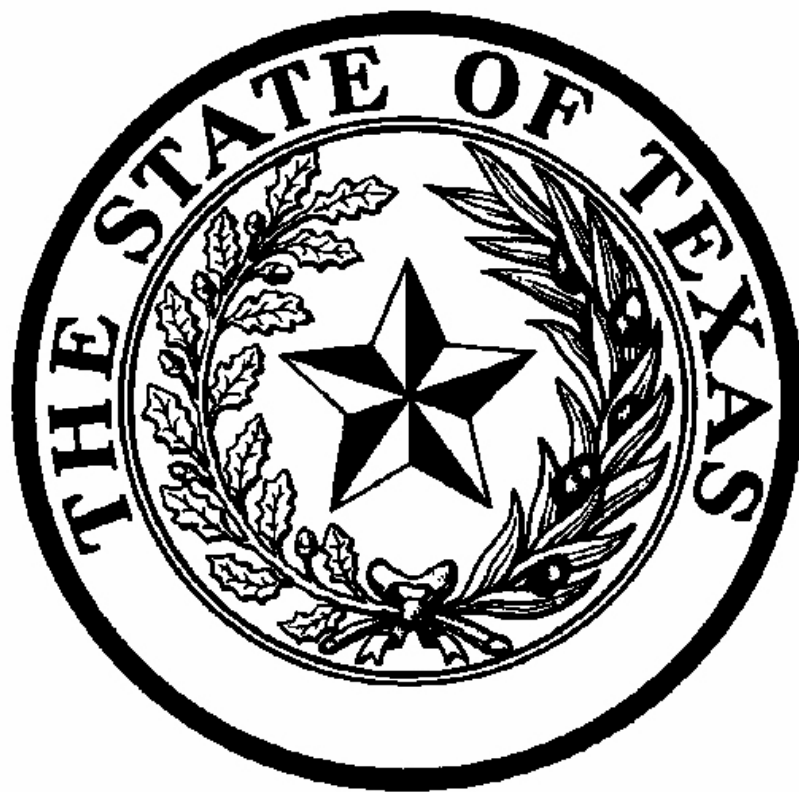

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The Texas Journal of Agriculture and Natural Resources publishes articles of recent research work, either descriptive or experimental in nature. Articles from the various disciplines of agriculture and natural resources will be published in the Journal subsequent to critical peer review and approval of the editorial board. Articles submitted for publication in the Journal are to report on work done in Texas by university personnel and their associates. No article is to be submitted for review if the manuscript is under consideration by another journal or magazine. Articles are to be written with critical research analysis but in a style that is easily understood by professional agriculturalists and resource managers. Publication charges are \$50.00 per journal page for ACT members and \$100 per page for non-ACT members (double the ACT member charge) with a minimum charge of \$150.00 per article.

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Lunar Influence on Post-Castration Performance of Baby Piglets

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ABSTRACT

Three farrowing studies were conducted at Tarleton State University focusing on post-castration performance of piglets. In all studies, half of the boar piglets were castrated with lunar influence when the *Farmer's Almanac* recommended and the remaining half when castration was not suggested. There was no difference ($P > 0.05$) in growth in the days following castration up to weaning in all three studies ($n = 115$). The results for 30-day nursery gains were inconclusive. There was a difference in the 30 days of nursery growth ($P < 0.05$) favoring castration against the *Farmer's Almanac* recommendations in the first study, while Study 2 favored castration with lunar influence during the 30 days of nursery growth ($P < 0.05$). There was no difference in treatments in Study 3. Piglets castrated with lunar influence maintained a body temperature more close to the ideal level in the first two studies while there was no difference in Study 3. Study 1 found no difference in healing score while Studies 2 and 3 favored castration with lunar influence.

KEY WORDS: piglets, castration, lunar influence, performance, healing

INTRODUCTION

In any animal agricultural enterprise, castration of males is likely to occur. The purpose of castration is to control unwanted reproduction and to control sexual behavior of males. Intact males often become aggressive and dangerous to their handlers and other animals, and tend to damage property and facilities. Some producers believe waiting to castrate their animals can enhance a faster rate of gain and increase feed efficiency. Conversely, other producers castrate the males at an earlier age, which is less stressful on the animal and handlers. Still, some producers strategically castrate their animals based on recommendations from the *Farmer's Almanac*. This concept is based on the theory that the position of the moon affects blood flow and ultimately the stress level on the animal.

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In the swine industry, the ideal time for castration to occur is debatable. Producers either castrate when time permits and labor resources are available, or they strategically plan when to castrate. In this case, the size of the pig at castration and the time for the process to occur are planned. One concept that has been passed is the theory of castrating when the *Farmer's Almanac* suggests. Prior research indicates that there are no differences in growth rates of piglets castrated while still nursing on the sow until weaning (Kielly et al. 1999). Yet, some pork producers continue to plan castration for their piglets when the *Farmer's Almanac* recommends.

Prior to castrating, some swine producers will review the *Farmer's Almanac* or Zodiac signs while others will not check this information and simply castrate their pigs. Stallings and Stallings (2010) wrote that "you should only castrate when the moon signs were in the 'thighs' of Sagittarius and going down." This is believed to trigger less bleeding because the signs are away from the heart and not moving in its direction. Under this assumption, if the signs were at Leo, or in the heart, the animal would bleed more profusely. Stallings' research was conducted in horses but the signs and bleeding are hypothesized to be influenced in all animals. It is suggested that the best time for castration is when the calendar shows Sagittarius (thighs), Capricorn (knee), Aquarius (legs), and Pisces (feet) in that order, respectively. Conversely, it is not suggested to castrate while the signs are Leo (heart) and when they are in the Pisces (feet) and going up. Days the Signs of the Zodiac are Scorpio (secrets) should also be avoided as it is associated with the scrotal region. Therefore, the objective of this project is to determine the effects post-castration performance as a result of Lunar influence (*Farmer's Almanac*). This project will determine if lunar castration results in improved growth and healing. A further determination will also be made to identify if castration with the *Farmer's Almanac* results in a lower body temperature because of less inflammation.

MATERIALS AND METHODS

One-hundred-and-fourteen male piglets were observed to determine if there was an effect on growth and healing based on the time of castration according to the lunar system. Crossbred swine were utilized in which the representation was of Hampshire, Yorkshire, Duroc, and Spotted Poland China influence. All research was conducted in a climate controlled farrowing house at the Swine Center at Tarleton State University in Stephenville, Texas. Farrowing crates within the house were 61 cm (2 ft.) by 213.4 cm (7 ft.) with an additional 61 cm on both sides for the piglets to escape the sow. Temperature was regulated to remain at a range of 16-27 °C for the sows. In addition, there was a heat lamp provided in each pen for the piglets to stay warm at approximately 32 °C. These practices were suggested by the National Pork Producers Council (1996). The heat lamp was elevated as the piglets grew. It was model e125012s120v 250w from Threede Lighting Technologies Inc. The heat lamp was hung so that the bottom of the heat radiation would meet at the top of the piglets' spines to maintain a temperature of 32 °C. The experiment was performed from September 2011 to September 2012 and included three separate farrowings and castrations. The first farrowing study occurred from September 2011 to October 2011 with an average temperature of 20 °C. The second farrowing study occurred from December 2011 to January 2012 when the average ambient temperature was 4.5 °C. The third farrowing study was observed in July-September 2012 with an average temperature of 30 °C (Geiger and Duncan 2012) even though sows and piglets were confined to the controlled temperature housing.

Immediately after parturition, each piglet within the litter was weighed for birth weight and identified. Piglets were then given 0.7 mL of Iron (200 mg/mL), 0.35 mL of Penicillin (300,000 IU/mL), and 0.4 mL of Draxxin (200 mg/mL) along with .25 mL of Exceed (Pfizer NY, NY). Litters were standardized with gilt piglets being moved from sow to sow so that each litter possessed similar numbers of piglets. At one week of age, piglets were given a 1 mL vaccination to protect against *Bordetella*, *Pasteurella*, and *Erysipelas* (BPE) as well as 2 mL of RespiSure (Pfizer NY, NY) to combat *Mycoplasma Pneumonia*. At 10 days of age, each piglet was given another vaccination of Iron, Penicillin, Draxxin, and Exceed. At 14 days of age, creep feeding was initiated (Table 1). Creep feed was monitored daily and fed ad libitum. After 21 days of age, 1 mL of BPE was given as a booster and 2 mL of the first Circovirus vaccine was administered.

Table 1. ACCO Showmaster Starter (Bannec) medicated with carbadox and pyrantel tartrate.

Item	Guaranteed Analysis
Crude Protein	23.00%
Lysine	1.61%
Crude Fat	5.00%
Crude Fiber	3.50%
Calcium	1.29%
Phosphorus	0.70%
Salt	1.00%
Sodium	0.60%
Selenium	0.3 ppm
Zinc	3,000 ppm
Chromium	200 ppb

Weight was obtained by utilizing a Fairbanks 1,100 scale. Rectal temperature was obtained with a ReliOn 9 Second Flex thermometer. Healing scores (one to six) were assigned independently by a three-person panel with the average of the panel being documented. In each farrowing study, the piglets were weighed the day prior to the first castration and randomly grouped into two categories. Each piglet's category was represented by a coin toss with heads being Treatment 1 and tails representing Treatment 2. Previously, the coin was flipped 20 times to insure randomization with heads occurring 11 times. The two categories represented those castrated when the Lunar signs suggested castration (Treatment 1) and those piglets castrated when the signs did not suggest castration (Treatment 2). Each litter was divided equally based on weight the day prior to the first castration in each farrowing study. Piglets were then randomly divided into each treatment group equally. There was no difference in weight ($P > 0.05$) between the two groups at castration.

In each farrowing study, the piglets were placed in a cart with solid flooring, and then moved to the scale area. Once the scale was tared to zero, a piglet was placed in the clean plastic container. Once the weight was recorded, the thermometer was inserted rectally. After approximately nine seconds for the thermometer reading, the temperature

was obtained and recorded. Furthermore, the incision was examined by the three-person trained panel for the purpose of assessing a healing score. The healing score was documented and the piglet was returned to the cart. The scale and plastic container was cleaned and tared for the next piglet to be weighed. Once the data for all of the male piglets in each litter were documented, all piglets were then returned to their respective sow.

At 24 to 28 days of age, the piglets were weaned from the sow and moved into the nursery in an “all in, all out” management system. Litters were kept together in nursery pens measuring 213 cm by 155 cm that were elevated 29 cm off the ground. All piglets were fed a commercial starter ration ad libitum. The nursery room was also climate controlled to remain at 30 °C. After 21 days in the nursery, the second Circovirus vaccine was administered (2 mL intramuscularly). After 30 days in the nursery, the piglets were weighed and then moved to the finishing floor. The data were obtained and compared for statistical analysis were as follows: body weight: one, two, three, six, eight, nine, and 10 days following the first group castration, adjusted 21-day weaning weight and 30-day end of nursery weight. Weights were obtained on the first three days post-castration to show differences in gains between treatments. Day six weight was obtained to show no difference in treatments prior to the second group’s castration on day seven. Weights on day eight, nine, and 10 were collected to compare gains between treatments following the second castration using day six weight as a starter to determine gains. Also, the body temperature was obtained the first two days post-castration and healing scores: one, two, three, and six days post-castration were recorded. Carroll et al. (2006) reported the effects of castration were most evident in the first 48 hours post-castration so body temperatures were obtained during those hours. Healing scores were obtained the first three days post-castration to examine the effect of lunar influence. Most piglets were not completely healed in the first three days so an examination on the sixth day was utilized to draw a healing score over a longer time period (Gleason 2012).

Castration Protocol (Farrowing Study I). Following birth, piglets were given six days to establish their natural order on the mammary system and then weighed on day six. The first farrowing study consisted of 27 piglets in Treatment 1 and 28 piglets in Treatment 2. Castration was performed on day seven for Treatment 2 and day 14 for the Treatment 1 in accordance with the *Farmer’s Almanac* 2011 (Geiger and Duncan 2010). Prior to castration of each piglet, they were sprayed with a commercial anti-septic (70% isopropyl alcohol) directly on the site of incision. Each piglet was castrated with a Swann-Morton size 12 scalpel utilizing a handheld cradle method of restraint (Quiverfull 2008). This is a widely accepted method within the swine industry. A new sterile scalpel was used on each piglet. The technician and handler wore and discarded sterile plastic gloves so that new gloves were used on every piglet. The incision was made 3 to 5 cm ventral to the anus in the lower scrotal region. The castration occurred using the National Pork Producers Council’s (1996) approved method of pulling the testicle free from the spermatic cord with the technician utilizing a sterile tissue to grasp the testicle. The wound was left open for proper drainage and any spermatic cord that did not separate freely was trimmed. This is a normal practice in the swine industry. The same castration techniques were utilized on Treatment 1 group of the boar piglets on the fourteenth day of life when the *Farmer’s Almanac* suggested castration.

Castration Protocol (Farrowing Study II). In the second farrowing study, each treatment group was represented by 15 piglets. Treatment 2 piglets were castrated at 14 days of life and the subsequent castration for Treatment 1 piglets occurred at 21 days of life, when the lunar signs changed and suggested castration for the Treatment 1 group. The same techniques that were utilized in the first farrowing study were also used in the second farrowing study.

Castration Protocol (Farrowing Study III). In the third farrowing study, the Treatment 1 group consisted of 14 piglets and was castrated at 12 days of life when the lunar signs were favorable for castration (Geiger and Duncan 2011). The Treatment 2 group which was comprised of 15 piglets was castrated at 19 days of life when the lunar signs changed and did not suggest castration. The castration techniques that were utilized in the previous farrowing studies were used in this farrowing study.

Weighing Protocol. Again, all piglets were weighed the day prior to the first castration. On the first day post-castration, all barrows and intact boar piglets representing both treatment groups were weighed with subsequent weights being obtained on the second, third, and sixth day. Following the subsequent castration on day seven for the remaining treatment group, both of the treatment groups were weighed on day eight, nine, and 10 based on the first treatment group castration day. Also after weaning, all piglets were weighed at day one in the nursery and after 30 days in the nursery to obtain the effects on nursery performance. In addition, an adjusted 21-day weaning weight was calculated from a weaning weight based on the day weaned.

Temperature Protocol. Prior to the piglets being carted from the farrowing crate, the thermometer was calibrated each day. This was achieved by filling a 250 mL beaker with water and ice. The mixture was then stirred for two minutes and covered with a piece of cardboard. After three minutes, the thermometer was inserted through the cardboard with the majority of the stem immersed in the ice bath without touching any part of the beaker. If the thermometer did not read 0 °C, it was not used. These methods for calibrating a thermometer are standard procedures suggested by the USDA FSIS (1995).

Once the weight was documented, while the piglet was still in the plastic container, the thermometer was inserted rectally by holding the piglet by the tail with one hand and inserting the thermometer with the other. The thermometer was left in the rectum until the “beep” signal from the thermometer sounded (approximately nine seconds). Rectal temperatures were obtained on the first and second day post-castration.

Healing Score Protocol. On the first day post-castration, all piglets that were castrated on the previous day were examined and given a healing score by three members of an expert panel. The scores had a range of one to six. Piglets that received a six possessed an open wound that was inflamed and pustular. For a piglet to receive a healing score of five, it possessed an open wound with no inflammation. A score of four was given to piglets that had a wound in which a scab began to crust over with a red ring around the incision with inflammation. A healing score of three was set for individuals that had a scab that was beginning to crust over with a light pink ring around the incision. Piglets that obtained a grade of two had a small scab with a wound that was almost healed. A healing score of one was considered to have a wound that was completely healed. Panel members independently scored each piglet and subsequently an average for each piglet

was calculated. Subsequent evaluations occurred on days two, three, and six on the piglets that were castrated in the initial castration. The second group castrated was examined for healing score on days eight, nine, 10, and 13 from the initial castration. Therefore, the data represented for healing scores was one, two, three, and six days post-castration because the second castration scores that were taken on days: eight, nine, 10, and 13 were the first three days post-castration and the sixth day after that treatment's castration. Therefore, this derived data for both groups to be analyzed was on days one, two, three, and six, following their individual castrations. The panel evaluated the wound based on the previously described scoring system which was approved by a licensed veterinarian (Gleason 2012) and determined the given value. Previously utilized healing scoring systems could not be found in the literature.

Statistical Analysis. In order to obtain results that were valid, repeatable, accurate, and were true representatives of the population, statistical analysis was conducted using SPSS V 19 (SPSS, Illinois 60611, USA 2009). ANCOVA univariate analysis was conducted on weight gains for days one through 10 post-castration with weight gain being the dependent variable, treatment as a fixed factor, and day as a covariate. For 21-day weaning weight and weight gains in the nursery, a *t*-test was utilized. For temperature results, the ANCOVA univariate analysis was applied with temperature being the dependent variable, treatment as the fixed factor, and day as a covariate. Healing score was analyzed with ANCOVA univariate analysis because the panel gave an average so the data was continuous. Healing score was the dependent variable, treatment as a fixed factor and day as a covariate. Results were considered significant if $P < 0.05$.

RESULTS AND DISCUSSION

Growth. After analysis, there were no differences between treatments for piglets from castration to weaning in all studies ($P > 0.05$). This was in agreement with Hay et al. (2003) and Carroll et al. (2006) which found castration to cause no reduction in growth for the first 48 hours post-castration. There were no differences between treatment groups' 21-day adjusted weaning weight for all studies ($P > 0.05$). This was in agreement with Kielly et al. (1999) as neither treatment group possessed a statistical difference from birth until weaning.

However, there was a significance ($P < 0.05$) shown in Table 2 for 30-Day Nursery Weight. Results were inconclusive where data suggested to castrate boars when the *Farmer's Almanac* does not suggest based on lunar influence in post-weaning growth for one study while data suggested to castrate with lunar influence in another study. There was no difference between treatment groups ($P > 0.05$) in Study 3.

The Treatment 1 group posted a 30-day nursery weight gain of 8.56 kg while the Treatment 2 group was heavier at 9.79 kg. Yet, the second study shows differing results following 30 days post-weaning. Piglets that were castrated based on when the *Farmer's Almanac* suggested posted 7.71 kg of gain compared to the Treatment 2 group at 6.07 kg ($P < 0.05$). Conversely, the mean values of weight gain following 30 days in the nursery, shown in Table 2, were not similar to either previous study.

Table 2. Mean Values for 30-Day Weight Gain for Piglets Castrated Based on Lunar Influence.

	Treatment 1		Treatment 2	
	n-value	Weight Gain, kg	n-value	Weight Gain, kg
Study 1	28	8.56 ^a	27	9.79 ^b
Study 2	15	7.71 ^a	15	6.07 ^b
Study 3	14	11.90 ^a	15	11.00 ^a

Means within a row with different superscripts differ $P < 0.05$

Body Temperature. Figure 1 depicts the mean values of temperatures for the piglets in the first farrowing study. At day two post-castration, there was a significance between the treatment groups ($P < 0.05$). The Treatment 1 group maintained a body temperature more close to the normal level of 39 °C. This is the normal body temperature and is in agreement with other researchers (Lammers et al. 2007; Mount 1959). The Treatment 2 group possessed a lower body temperature on both days with the second day post-castration displaying a reduction in temperature, yet in all situations piglets did not require medical attention according to a consulting veterinarian (Stevenson 2012).

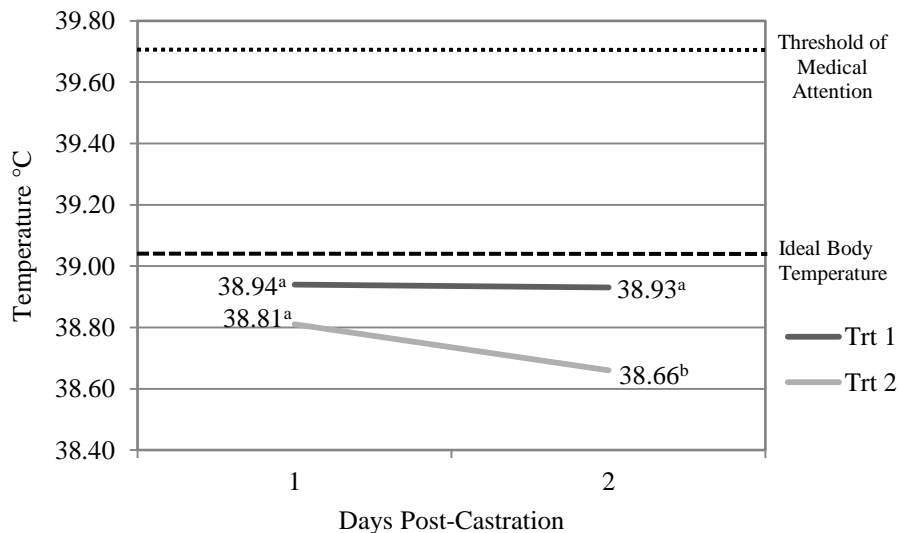


Figure 1. Mean Values for Body Temperature for Piglets Castrated Based on Lunar Influence (Study 1) Means within a day with different superscripts differ $P < 0.05$.

Figure 2 shows the mean values of body temperatures for the piglets in the second farrowing study. There was a significance between the treatment groups ($P < 0.05$) and a treatment by day significance ($P < 0.05$) at day one. Treatment 1 group possessed a body temperature slightly lower than the ideal body temperature on the first day while the Treatment 2 was slightly elevated. Furthermore, Treatment 1 was significantly lower in body temperature yet neither was at a level that required medical

attention. Yet, on the second day post-castration both groups were close to the ideal body temperature.

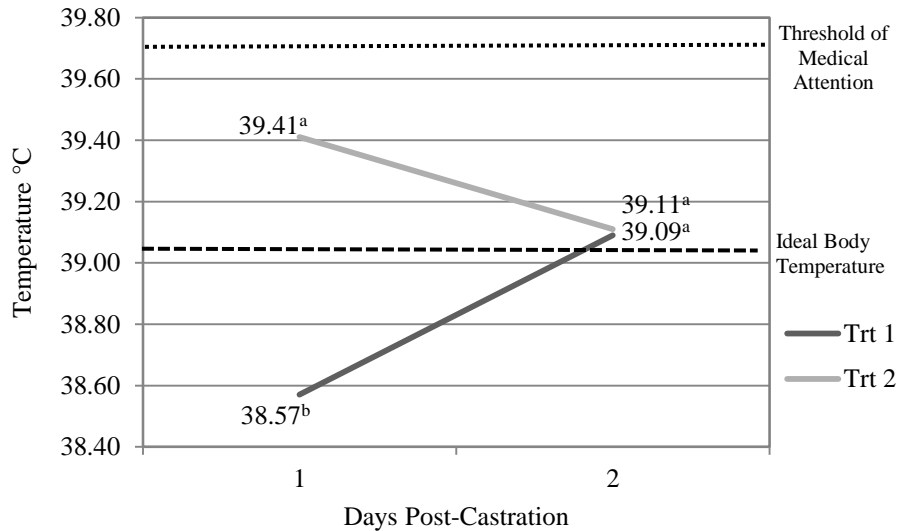


Figure 2. Mean Values for Body Temperature for Piglets Castrated Based on Lunar Influence Study 2) Means within a day with different superscripts differ ($P < 0.05$).

Figure 3 depicts the mean values of temperatures for the third farrowing study. There was no significance between the treatment groups ($P > 0.05$). On both days, the treatment groups were very close to the normal body temperature of 39° C and were not statistically different ($P > 0.05$).

Healing Score. Figure 4 shows the mean values of healing scores for the piglets castrated by lunar influence in the first farrowing study. There was no significance between treatment groups ($P > 0.05$) but there was a day significance ($P < 0.05$). Although there were no differences between the treatment groups on any day, both groups were significantly lower as each day passed to the next. This was reflected by the natural healing process.

Figure 5 depicts the mean values of healing scores for the piglets castrated by lunar influence in the second farrowing study. Treatment 1's mean value was considerably lower the first day post-castration ($P < 0.05$). However, treatment groups were similar in the remaining days for healing scores with inconsistency between the treatment groups for various days.

Figure 6 shows the mean values of healing scores for the piglets castrated by lunar influence in the third farrowing study. There was a treatment by day effect on healing score ($P < 0.05$) with the treatment significance being ($P < 0.05$). Treatment 1 was consistently lower than the Treatment 2 group for days one, two, and six. However at six days post-castration, Treatment 2 groups mean value remained the same (2.14) as the day three healing score and did not lower, while the Treatment 1 group healed and possessed a lower mean value (1.27).

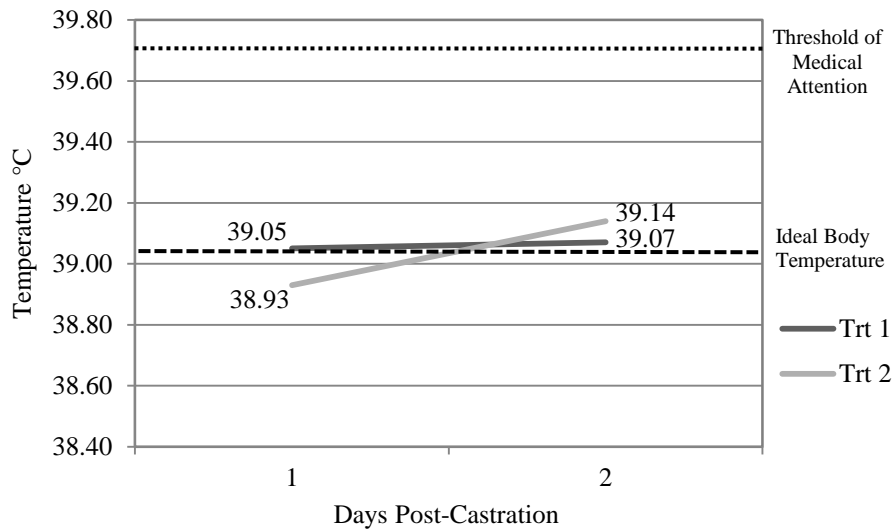


Figure 3. Mean Values for Body Temperature for Piglets Castrated Based on Lunar Influence (Study 3).

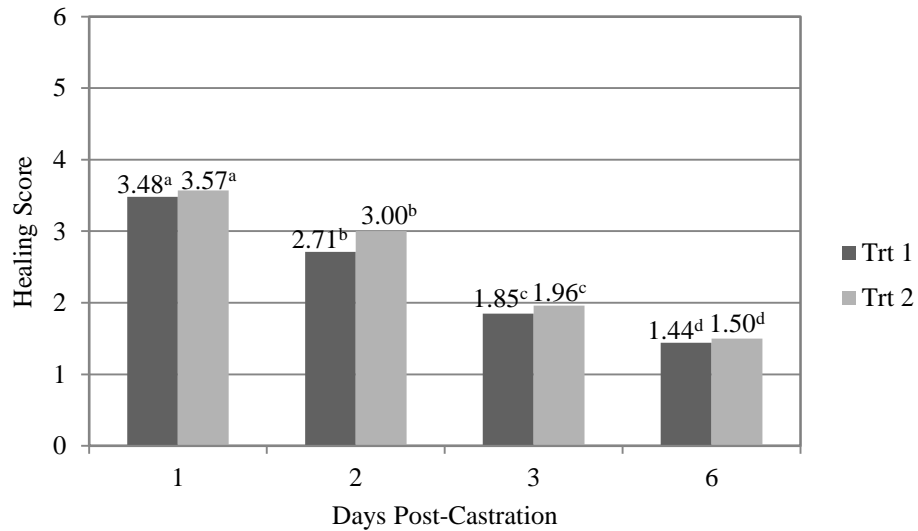


Figure 4. Mean Values of Healing Score for Piglets Castrated Based on Lunar Influence (Study 1).

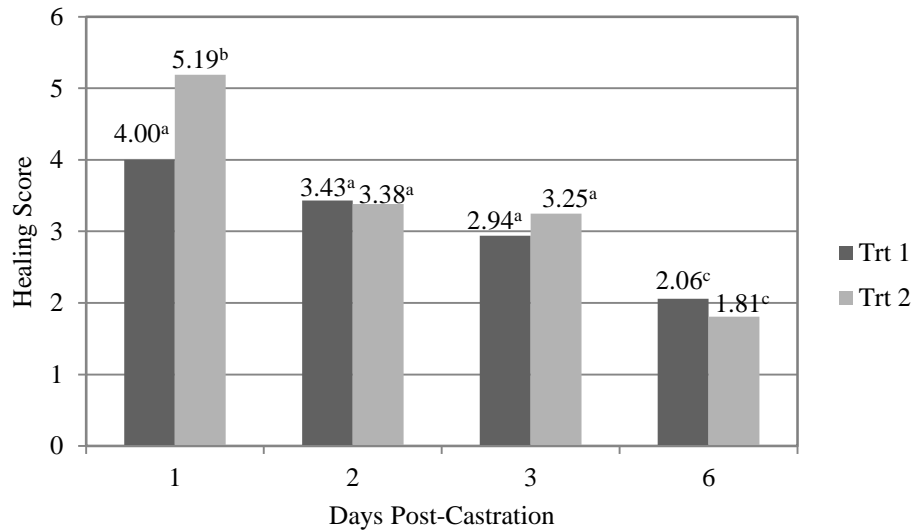


Figure 5. Mean Values of Healing Score for Piglets Castrated Based on Lunar Influence (Study 2). Means within a day with different superscripts differ ($P < 0.05$).

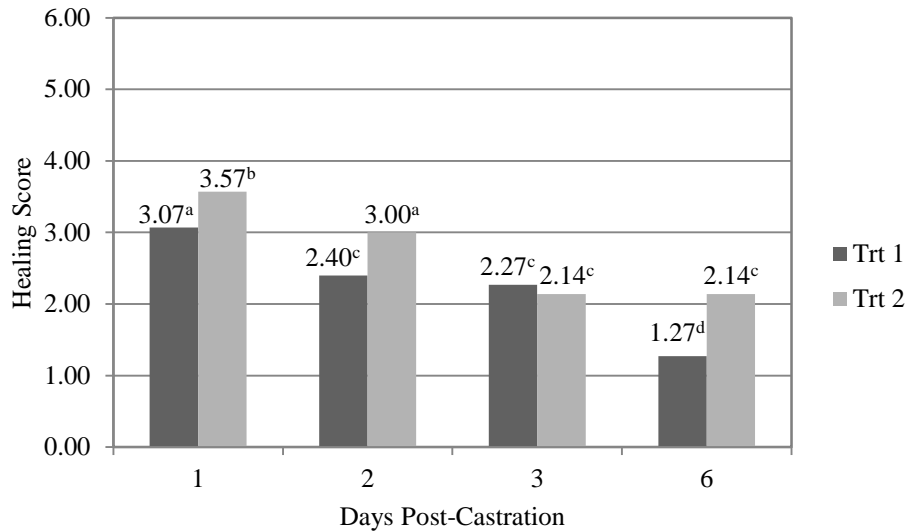


Figure 6. Mean Values of Healing Score for Piglets Castrated Based on Lunar Influence (Study 3). Means within a day with different superscripts differ ($P < 0.05$).

CONCLUSION

The hypothesis stated there will be no difference in growth of piglets castrated when the *Farmer's Almanac* suggests compared to those that were castrated against the *Farmer's Almanac* recommendations. This hypothesis failed to be rejected. There was no

statistical evidence in any replication of the piglets' growth rate for differences compared on the first 10 days post-castration and at weaning. However, after 30 days in the nursery, all three studies generated conflicting results. Therefore, more research should be conducted to determine if a relationship may exist during the nursery growth period.

The hypothesis that stated there will be no difference in body temperature of those piglets castrated when the *Farmer's Almanac* suggests castration compared to those castrated when the *Farmer's Almanac* does not advocate castration. Also, this hypothesis failed to be rejected. Although there was noted a significance in the first two replications, the temperatures were still close to the normal body temperature. Therefore, none of the temperatures required medical attention. In the third farrowing study, there were no differences ($P > 0.05$).

The hypothesis that stated there will be no difference in the length of time required for the incision to heal in those piglets that were castrated with the recommendations of the *Farmer's Almanac* versus those that were castrated when the *Farmer's Almanac* did not recommend castration. This hypothesis was rejected. The first study did not find a significance between treatments ($P > 0.05$). Yet, in studies two and three, evidence showed that piglets that were castrated with lunar influence began to heal earlier than those piglets that were not and the mean value was lower on the first day post-castration. In the first two studies, the piglets that were castrated with lunar influence were older and were castrated a week later in life but still began to heal at an earlier day than their littermate comparisons. In the second farrowing study, the first day post-castration there was a difference between treatment groups ($P < 0.05$) favoring castration with lunar influence. The third farrowing study was conducted with males that were castrated with lunar influence at 12 days of age while their counterparts were castrated without lunar influence at 19 days of age. There was a significance ($P < 0.05$) favoring castration when the *Farmer's Almanac* suggests based on lunar influence. The major difference between treatment groups occurred in healing from days three to six.

Recommendations to continue this study would be to determine if lunar influence has an effect on nursery performance. Another recommendation is to perform this study on pigs that are larger and out of the nursery to determine if lunar influence has a greater effect on growth as the pig increases in size. Along with this, the rate of healing might increase with lunar influence as the pigs increase in size because as the piglet grows the stress from castration will increase as body size increases (Miller and Ingram 1991). The final recommendation is to perform a similar study on another species.

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Intake and Nutritional Quality of Different Types of Onions by Lambs

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ABSTRACT

Onions (*Allium cepa*) may reduce some nutritional deficiencies in ruminants. In addition, onions reportedly contain L-cysteine which may bind with the toxic compound in bitterweed (*Hymenoxys odorata*) to create a less toxic compound. We compared intake, nutritional quality, and L-cysteine levels among the types of onions. Red, yellow, or white onions were fed to Rambouillet lambs in individual pens in April, July, and November. A fourth treatment only received the basal diet of alfalfa (control). Intake was monitored daily. Weights were taken at the beginning and at the end of the trial. Packed cell volume was measured in each feeding trial to assess anemia. During the April trial, serum metabolite levels, that are indicative of toxicosis, were also measured. Intake of onions and alfalfa were similar ($P > 0.05$) among treatments. Packed cell volume was lower ($P < 0.05$) for lambs fed alfalfa alone in April, but was similar among all treatments in July and November. L-cysteine levels were undetected when measured in April. Serum metabolite levels remained within normal levels. Nutritional quality was also similar among types of onions. Lambs readily consumed onions and avoided toxicosis even when onions made up 100% of the diet.

Key Words: *Hymenoxys Allium*, onions, cysteine, supplementation

INTRODUCTION

Onions that do not enter into the human food chain are sometimes fed to livestock. In West Central Texas, there is some interest in feeding onions to sheep as a supplemental feed and to reduce the likelihood of bitterweed (*Hymenoxys odorata*, DC.) toxicosis. Bitterweed is considered one of the most problematic plant species in western Texas (Hardy et al. 1931), especially in the Edwards Plateau region. Since the early 1920's, bitterweed has been recognized as being poisonous to sheep (Sperry 1949) and was reported to be the most serious toxic plant problem faced by sheep producers in Texas (Calhoun et al. 1980).

The toxin in bitterweed is a sesquiterpene lactone known as hymenoxon (Kim et al. 1975; Ueckert and Calhoun 1988). Symptoms of hymenoxon poisoning include bloat, central nervous system recession, termination of rumen activity, kidney damage, liver

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damage, and eventually death (Rowe et al. 1973; Witzel et al. 1974). Hymenoxon conjugates in the rumen with sulfhydryl groups, forming a less toxic compound (Calhoun et al. 1989). The amino acid L-cysteine contains sulfur and may provide sulfhydryl groups for conjugation in the rumen. Rowe et al. (1980) dosed sheep with cysteine, increasing the survival rate up to 12-fold over sheep that were not dosed. Onions (*Allium cepa*, L.) contain moderate to high levels of cysteine. Some report that sheep will readily consume onions when fed a 100% onion diet and can perform as well on the onions as on a grain diet (Knight et al. 2000), while others caution that onion toxicity can occur with over-ingestion causing anemia in animals (Cheeke 1998).

Different onion types differ in phenolic content, antioxidant, and anti-proliferative activities (Yang et al. 2004). Changes in the concentration and total amount of flavor precursors like S-alk(en)yl-L-cysteine sulphoxides during the growth of an onion should affect the flavor and potentially the level of L-cysteine in onions (Lancaster et al. 1983). This may explain why some report ruminants will readily consume onions without any adverse effects (Campbell et al. personal communication) while others report that ruminants are reluctant to consume onions (Bundick 2008). Campbell et al. also reported that onions reduced the likelihood of bitterweed toxicosis, while Bundick (2008) showed that onions did not offer any protection from bitterweed toxicosis. Accordingly, this study assessed intake of different onion types by lambs, differences in nutritional quality, and L-cysteine levels between types of onions. The study also determined if an intake of onions resulted in anemia or soft tissue damage from toxicosis.

MATERIALS AND METHODS

This study consisted of three trials that were conducted at the Angelo State University Management Instruction and Research (MIR) Center. Trial 1 was conducted in April 2009. Trial 2 was conducted in July 2009 and Trial 3 was conducted in November 2009. During each trial, lambs were randomly allocated to treatments, with each treatment fed a different type of onion (red, yellow, or white).

Forty recently weaned Rambouillet lambs (46.6 kg) were randomly allocated to four treatments (n = 10/treatment). The lambs were separated into individual pens (1 X 1.5 m) and allowed 5-10 days for pen adjustment. Alfalfa pellets were fed at 2.5% BW to meet the animal's maintenance requirements as specified by the National Research Council (2007). Lambs received fresh water and a calcium/phosphorus mineral *ad libitum*, and excreta were removed weekly. The research protocol was approved by the Angelo State University Animal Care and Use Committee.

After the adjustment period, lambs were fed onions daily for 22 days. The first treatment was supplemented with red onions, the second with white onions, the third with yellow onions, and the fourth treatment served as the control group receiving only alfalfa pellets. During the 22-day study, the onions were chopped by hand and offered from 1,300 to 1,400 each afternoon. The amount of onions fed began at 75 g. Once an individual lamb consumed all onions for two consecutive feeding bouts, the amount was increased by 25 g until the point of refusal. All the lambs received the 2.5% BW alfalfa after the onions had been offered to meet maintenance requirements.

Any changes in weight, blood serum levels, and packed cell volume (PCV) were recorded during this experiment to monitor for any signs of toxicosis. Serum metabolite levels and packed cell volume were monitored at days 0, 10, and 22. Changes in serum levels indicative of toxicosis include levels of serum aspartate transaminase (AST), blood

urea nitrogen (BUN), gamma glutamyltransferase (GGT), creatinine, and bilirubin (Cornelius 1989; Cheeke 1998). Blood was collected via jugular vein-puncture, serum extracted by centrifugation (3,000 rpm for 20 minutes), frozen (-80 °C), and sent to the Texas Veterinary Medical Diagnostic Lab in College Station, Texas, for chemical analysis. Packed cell volume was measured as a percent of red blood cells (RBC) by transferring fresh blood in capillary tubes 30 minutes after collection, then centrifuging the tubes for 15 minutes and measuring the red blood cells with the scale affixed to the centrifuge. All lambs were weighed at the beginning and end of the study to assess any variation in weight among treatments.

Subsamples of each type of onions were analyzed for crude protein, digestibility, Acid Detergent Fiber, Neutral Detergent Fiber, Net Energy for maintenance (NEM), and moisture content by Dairy One in Ithaca, New York. Another subsample of each onion was sent to NP Analytical Labs in St. Louis, MO for L-cysteine levels.

In July 2009, a second trial was conducted and consisted of 32 ($n = 8/\text{treatment}$) recently weaned Rambouillet lambs (36.5 kg). The purpose of the second trial was to determine if the trends observed in the first trial, particularly the differences in PCV, would be repeated. The treatment groups and feeding protocol were identical to Trial 1. Serum levels were not measured in Trial 2, but packed cell volume (PCV) was measured. Blood samples were collected and centrifuged to measure PCV levels using the same methods as in the first trial. L-cysteine levels of the onions in Trial 2 were not measured because levels were undetectable in Trial 1; however, nutritional analysis was measured. Intake of onions and alfalfa were measured throughout the study. Lambs were also weighed at the end of the feeding period to evaluate weight gain/loss.

In November 2009, freshly weaned Rambouillet lambs (33.4 kg) were fed the three types of onions in the same manner as the first two trials. Lambs were randomly allocated to one of four treatments, which corresponded with the first two trials. Initially, the trial consisted of 40 lambs ($n = 10/\text{treatment}$), but one died of natural causes six days into the trial. This trial differed from the first two trials in that the amount of basal ration was reduced systematically throughout the trial until lambs were consuming a diet that consisted of 100% onions.

For the first 14 days, alfalfa was offered at 2.5% BW after onions were offered. Onions were offered initially at 75 g, and once complete onion consumption was achieved, the onions were increased by 25 g each subsequent day. During days 15-21, alfalfa was decreased to 2% BW. Alfalfa levels were again decreased on days 22-28, 29-35, and 36-42 to 1.5%, 1%, and 0%, respectively. The control group received 2.5% BW alfalfa throughout the duration of the trial.

Intake of onions and alfalfa were monitored daily along with packed cell volume. Packed cell volume was evaluated on days 0, 17, 24, 31, 38, and 45. Blood was collected via jugular vein-puncture, placed in capillary tubes then centrifuged and measured to obtain the packed cell volume. Body weight was measured at the beginning and end of the trial.

This study design was a completely randomized design. Differences between the types of onions were assessed using repeated measures analysis of variance. The individual lambs nested within treatments served as replications, and the day of observation was the repeated measure. Means were separated using Tukey's LSD where $P < 0.05$ was considered significant. Each trial was analyzed separately using the statistical package JMP (SAS Institute 2007).

RESULTS

April 2009. In Trial 1, the intake of onions and alfalfa was similar ($P > 0.05$) among treatments (Table 1). Lambs were hesitant to consume onions initially, but intake increased for all three treatments across the days of feeding. By the end of the study, intake varied among treatments (treatment by day interaction; $P < 0.05$) (Fig. 1). By the end of the trial, the lambs were consuming on average $2.4 \text{ g} \cdot \text{kg}^{-1} \text{ BW}$ of onions per day in each treatment.

Table 1. Average intake ($\text{g} \cdot \text{kg}^{-1} \text{ BW}$) of onions and alfalfa by lambs receiving different onion types in April, July, and November.

Food Item	Treatment			SEM
	Red	White	Yellow	
April				
Onions	0.9	1	1.2	0.2
Alfalfa	10.6	11.1	11.2	0.3
July				
Onions	1.4	1.2	1.2	0.3
Alfalfa	25	24.7	21.9	1.8
November				
Onions	20.6	23.4	21.8	1.3
Alfalfa	16.3	16.3	16.3	0.01

¹The amount of onions offered was increased over the trial until onions made up 100% of the diet.

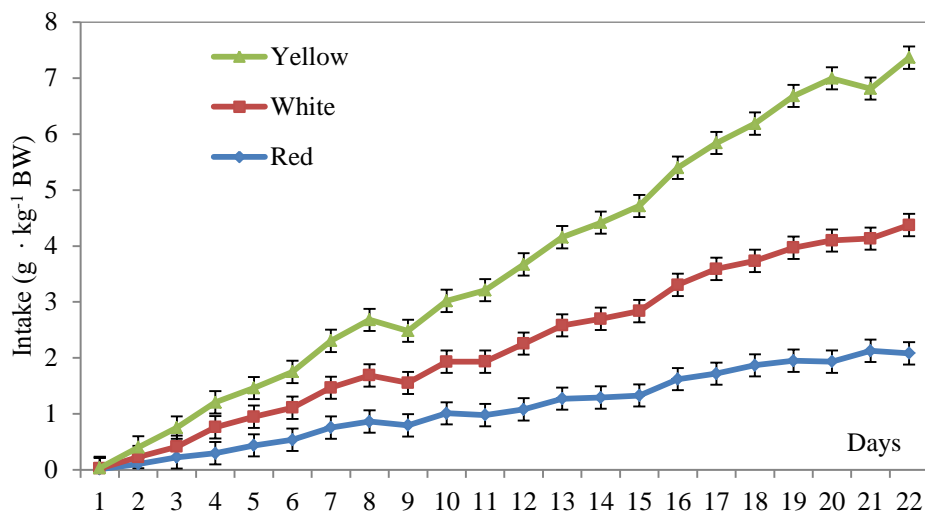


Figure 1. Average daily onion intake ($\text{g} \cdot \text{kg}^{-1} \text{ BW}$) by lambs during April. The treatment X day interaction differed ($P < 0.05$).

Some serum levels differed among treatments and days, but remained within normal ranges for healthy animals (Tables 2 and 3). Creatinine levels differed between

the control and the treatment receiving yellow onions. Gamma glutamyltransferase (GGT) levels from Day 1 collection period differed from the Day 11 and Day 22 collections (Table 3).

Table 2. Average serum metabolite levels of lambs within treatments in the April trial.

Serum Metabolite	Treatment				SEM	Normal Range ¹
	Red	White	Yellow	Control		
GGT (U/l)	73.7	65.1	61.2	62.1	4.8	34-82
AST (U/l)	114.8	112.5	89.9	110.1	6.9	51-130
Creatinine(mg/dl)	0.74 ^{ab}	0.72 ^{ab}	0.64 ^b	0.74 ^a	0.03	03-1.3
BUN (mg/dl)	24	23.5	22.3	24.8	0.8	12-32
Glucose(mg/dl)	58.1	60.4	59.8	61	2.3	58-109

^{ab} Superscripts that differ are significantly different ($P < 0.05$)

¹Normal range as reported by the Texas Veterinary Medical Diagnostic Laboratory, College Station, Texas.

Table 3. Serum metabolite levels of lambs in April by collection day. Data were pooled across all treatments because of a lack of treatment effect ($P > 0.05$).

Serum Metabolite	Collection Day		
	Day 1	Day 11	Day 22
GGT (U/l)	73.9 ^a	60.7 ^b	62.0 ^b
AST (U/l)	120.2 ^a	118.0 ^a	82.3 ^b
Creatinine(mg/dl)	0.6 ^c	0.8 ^a	0.7 ^b
BUN (mg/dl)	23.8 ^b	21.6 ^c	25.6 ^a
Glucose(mg/dl)	76.7 ^a	58.6 ^b	44.1 ^c

^{abc} Superscripts that differ are significantly different ($P < 0.05$)

Regardless of their treatment diet, none of the lambs in the April trial exhibited signs of anemia. Packed cell volume counts were similar among lambs fed either red, white, or yellow onions and remained within the normal range for healthy animals (Table 4). When compared to the control treatment, PCV was higher ($P < 0.05$) for lambs receiving onions. Weight change was similar ($P > 0.05$) among treatments (Table 5).

July 2009. In July, alfalfa and onion intake were similar ($P > 0.05$) among the three treatments (Table 1). Once again, the lambs were initially reluctant to consume onions, but intake for all three treatments increased across the days. By the end of the study, intake differed by treatments (treatment by day interaction differed; $P < 0.05$) (Fig. 2). Lambs in the onion treatments were consuming on average $3.9 \text{ g} \cdot \text{kg}^{-1} \text{ BW}$ of onions by the end of the trial.

Table 4. Packed cell volume (PCV) for sheep supplemented with either onions or no supplement in April, July, and November.

Treatment	Packed Cell Volume	SEM
April		
Red	37.9	0.9
Yellow	38.4	0.9
White	38.3	0.9
Control	35.1	0.9
July		
Red	35.2	2.3
Yellow	29.5	2.3
White	29.9	2.3
Control	34.3	2.3
November		
Red	37.1	0.8
Yellow	34.8	0.8
White	36.8	0.8
Control	37.4	0.8

Serum constituents were not measured in Trial 2 because all metabolites remained within normal levels in April. Packed cell volume counts were similar ($P > 0.05$) and within the normal range among all treatments in July (Table 3). Weight change was also similar ($P > 0.05$) among treatments (Table 5).

Table 5. Average weight (kg) for lambs supplemented with either onions or no supplement in April, July, and November. Weights were taken at the beginning and end of each trial.

Treatment	Beginning	End	SEM
April			
Red	45.7	43.5	3.2
White	46.1	44.0	3.2
Yellow	45.8	43.8	3.2
Control	46.5	44.5	3.2
July			
Red	37.3	33.8	2.4
White	34.3	29.2	2.4
Yellow	35.2	30.0	2.4
Control	39.1	36.0	2.4
November			
Red	33.0 ^a	29.2 ^b	1.1
White	32.6 ^a	27.3 ^b	1.1
Yellow	31.9 ^a	27.0 ^b	1.1
Control	36.2 ^a	36.2 ^a	1.1

^{ab} Superscripts that differ are significantly different ($P < 0.05$)

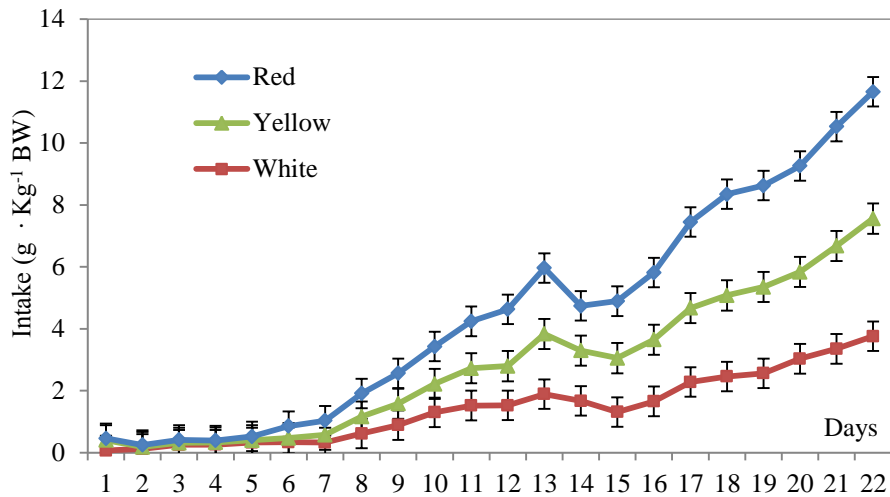


Figure 2. Average daily onion intake by lambs (g · kg⁻¹ BW) during July.

November 2009. In Trial 3, the intake of onions and alfalfa were similar ($P > 0.05$) among all treatments (Table 1). As the alfalfa was decreased, onion consumption increased to meet the nutritional demands that the alfalfa had filled (Fig. 3). The treatment X day interaction did not differ ($P > 0.05$). By the end of the trial, the lambs were consuming on average 49.4 g · kg⁻¹ BW of onions per day.

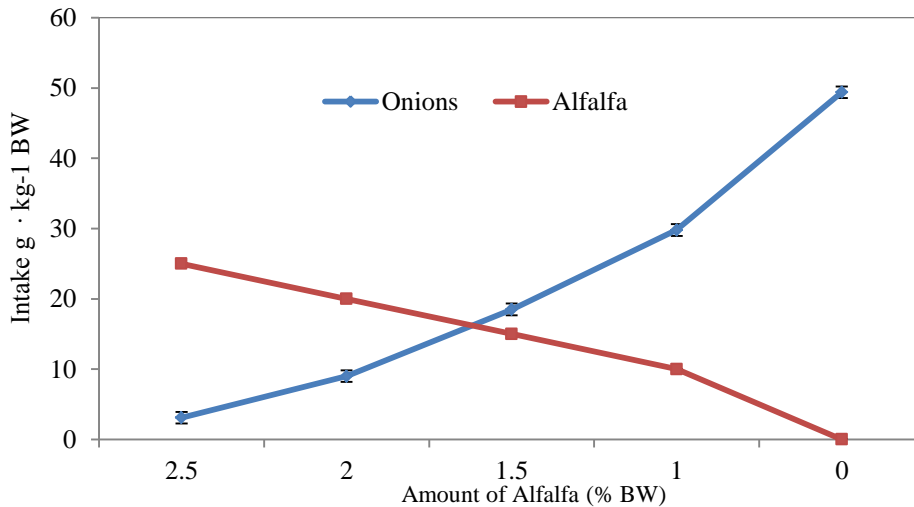


Figure 3. Onion and alfalfa intake (g · kg⁻¹ BW) of lambs for November.

Packed cell volume for Trial 3 was similar ($P > 0.05$) among all treatments (Table 4). The packed cell volume increased from initial collection to the final collection period during the trial (significant day of collection; $P < 0.05$) (Fig. 4). Weight change was also similar ($P > 0.05$) among the onion treatments with an average loss of 1.4 to 1.8 kg; the control maintained a constant weight throughout the trial (Table 5).

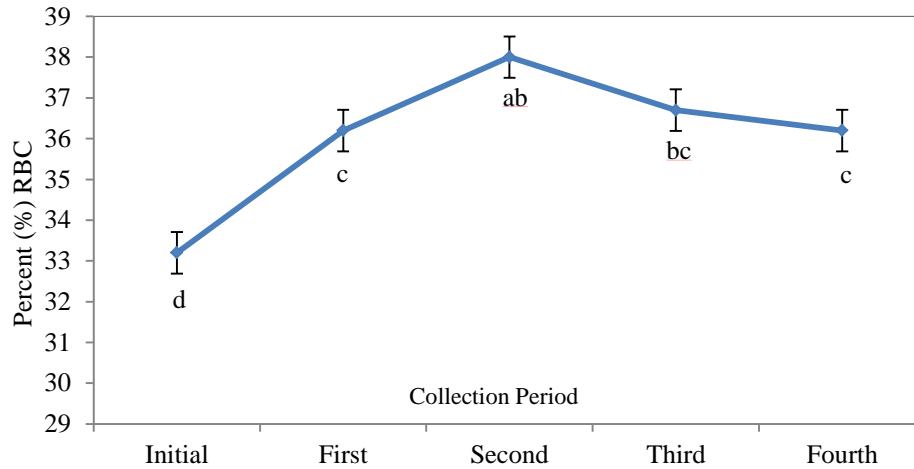


Figure 4. Packed cell volume (% RBC) by collection period for Trial 3.
^{abcd} Superscripts that differ are significantly different

Nutritional Analysis. Onions in all three trials revealed no nutritional differences between the three types that were offered for any of the variables measured (Table 6). Onions appear to be a moderately to highly nutritious supplemental feed.

Table 6. Nutritional analysis of the three onion types for the spring, summer, and winter trials.

Nutrient	April			July			November		
	Red	White	Yellow	Red	White	Yellow	Red	White	Yellow
Crude Protein (%)	14.0	13.4	11.5	8.6	11.3	9.3	15.2	10.8	12.7
NDF (%)	13.3	10.4	9.5	19.6	17.5	14.8	13.3	7.7	14.1
ADF (%)	9.7	9.9	8.4	14.9	13.8	11.0	10.0	6.7	12.0
NEM, (Mcal/lb)	0.88	0.89	0.90	0.84	0.85	0.87	0.88	0.91	0.87

CONCLUSION AND DISCUSSION

Results of this study indicate that lambs have no clear preference for any one of the three types of onions (red, yellow, and white) used in this study. By the end of the spring and summer trials, intake differed among types of onions (treatment by day interaction differed). In the spring trial, intake was highest of yellow onions, followed by white and red onions. For the summer trial, intake was highest for red onions, followed by yellow and white onions. Thus, there was no clear pattern of preference for one type of onion over the others. Nutritional quality of the three types was also similar in all three trials. Lambs quickly adapted to eating onions and readily consumed onions in all three trials without exhibiting any signs of toxicosis or anemia.

Toxicity from onions may have been avoided in this study because lambs were introduced slowly to onions over 14 days. We began by feeding small amounts (75 g) and increasing the amount offered slowly ($25 \text{ g} \cdot \text{hd}^{-1} \cdot \text{day}^{-1}$). Digestive physiology can be altered through exposure to poisonous plants early in life to the point that ruminants can avoid toxicosis. This observation was first illustrated by Distel and Provenza (1991). Goats, at six weeks of age, were fed blackbrush (*Coleogne ramosissima* Torr.) daily. Goats introduced to blackbrush early in life consumed 95% ($P < 0.01$) more blackbrush than naïve goats, were more efficient at digesting blackbrush, and excreted more uronic acid apparently because of an increased ability to detoxify the tannins in blackbrush.

Recent research with redberry juniper suggests that goats may be adapting to the toxic monoterpenoids in the plant as well (Bisson et al. 2001; Ellis et al. 2005; Dunson et al. 2007). These studies exposed goats to juniper in a pen situation for 14 days to allow for adaptation to the monoterpenoids in the plant. Goats typically increased intake up to 30% of the diet without experiencing any adverse effects from toxicosis. It is unclear if adaptation to toxic compounds in onions occurred in this study. Furthermore, the mechanism of the detoxification of onions is unclear.

In many other regions of the U.S., cull onions are used as a supplemental feed (McBride 2004). Onions reportedly range from 9-13% crude protein, 83-90% TDN, 0.35% Ca, 0.40% P, and 0.97 NE_m with a water content of 90% (Lardy and Anderson 2003). The onions in this study had crude protein levels ranging from 8-15%, NE_m from 0.84-0.91%, and 77-81% TDN, with moisture content of 90%. Calcium and phosphorus were not measured in this study. Ruminants need around 6-8% crude protein to maintain a healthy rumen, which onions easily could supply especially during winter when forage quality is limited.

Onions are apparently high in L-cysteine. We attempted to measure L-cysteine levels in the onions in April, but levels were undetectable. Dosing with L-cysteine has been shown to increase bitterweed intake while allowing animals to avoid hymenoxon-induced toxicosis (Rowe et al. 1980; Calhoun et al. 1986). Given onions' apparent levels of L-cysteine, others have suggested that onions may be an effective supplement to attenuate bitterweed toxicosis. In a preliminary trial, serum metabolite levels that are indicative of toxicosis were lower ($P < 0.05$) in lambs supplemented with onions (Campbell et al. unpub. data). Conversely, Bundick (2008) reported that supplementation with onions did not improve intake. Reasons for the differences in results remain unclear. However, Campbell et al. was able to get sheep to consume a diet consisting of 75% onions while Bundick (2008) was not.

Bundick (2008) also supplemented sheep with soybean meal which is high in L-cysteine in an attempt to increase the intake of bitterweed. Intake of bitterweed did not increase with soybean meal supplementation. Conversely, Coffman (2009) used four high-protein feeds (soybean meal, soybean meal/dried distillers grain, cottonseed meal, and cottonseed meal/dried distillers grain) to increase bitterweed intake. Supplementation with soybean meal increased intake of bitterweed while apparently allowing sheep to avoid bitterweed toxicosis.

Based on the results of this study, onions could serve as a viable supplement for sheep. Sheep readily consumed onions, but lost some weight when fed a diet of 100% onions. All three onion types are nutritious, and if intake is increased slowly, onion toxicity should not occur.

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Effects of Wet Aging and Temperature on Warner-Bratzler Shear Force, Sensory Characteristics, and Microbial Shelf-Life of Pork Loin Chops

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ABSTRACT

Twelve paired bone-in loins (IMPS# 410) were used to investigate effects of wet aging and temperature on Warner-Bratzler shear force (WBSF), cook loss, sensory characteristics, and microbial shelf-life. Loin chops (n = 120) were assigned randomly to two temperature groups (1.1 °C and 3.3 °C) and wet aged 0, 3, 6, 9, and 12 days. Higher WBSF values were reported for days 0, 3, and 9 (P < 0.05) than days 6 and 12. Microbial growth increased as days of aging increased. Juiciness, initial tenderness, and flavor increased as days of aging increased. These findings suggest wet aging pork loin chops can lead to improvements in product quality for the consumer.

KEY WORDS: shear force, wet aging, microbial, pork

INTRODUCTION

Addressing the problem of inconsistent meat palatability and the requirements for food safety are of great importance to the meat industry. Consumer dissatisfaction due to sensory attributes will be solved when unacceptable variations in meat sensory characteristics are minimized. Food safety will be improved when shelf-life attributes are identified and interventions are in place to prevent adulteration of products and microbial growth. Pork is the most widely consumed protein at 40% worldwide (USDA 2008) and is the third most popular in the United States. Pork producers struggle with in providing consumers with a uniform, highly palatable and wholesome product. Tenderness has been identified as one of the more influential attributes in determining consumer satisfaction of pork retail products (Kannan et al. 2002). Identifying consumer perceptions of tenderness and juiciness as well as establishing methods to assure wholesome products are important attributes for producers, consumers, and the scientific community.

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Beef products can undergo wet or dry aging to increase overall palatability. In the beef industry, aging has provided producers with an alternative for marketing their products based upon quality and uniformity. Aged beef products tend to have a more pungent and unique flavor profile. Unlike beef, the pork industry has not achieved the same popularity of dry or wet aging techniques. Methods utilized for aging, duration of aging periods, or temperatures during the aging periods have yet to be explored in the pork industry due to production costs, operational efficiencies, and variation in the chemical and physical properties of pork. Rancidity in pork products may occur more often due to fat types and diet sources of fats causing difficulties for aging pork without exceeding current industry standards (Koochmaraie 1992).

Unlike dry aging, controlled humidity and air velocity are not required for proper wet aging. Aging in vacuum packages substantially reduces weight losses during aging by up to 50% and reduces trim losses on the exterior of cut surfaces of beef products (Parrish et al. 1969). The rate of postmortem meat tenderization differs among species. According to Dransfield (1994) and Koochmaraie (1995), 80% of tenderization occurs in about five days for pork and 14 days for beef in order to reach the same degrees of tenderization. Differences in meat tenderness are related to differences in amounts of connective tissue within the muscles, formation of collagen cross-linkages in connective tissue components of muscles, contractile states of the myofibrils in the muscles when rigor bonds are formed, amounts and distribution of marbling within muscles, and extent of postmortem muscle proteolysis that occurs in muscles during aging processes (Smith et al. 1978).

However, pork quality can be enhanced through further research regarding maximizing shelf life and aging effects and how these traits impact palatability. This will require determining processes that affect meat palatability and perhaps more importantly adopting additional methods to assure tenderness, juiciness, and acceptable shelf life of pork products. Therefore, the objective of this study is to determine the impact of storage temperature and wet aging on tenderness, sensory characteristics, and shelf-life of pork loin chops.

MATERIALS & METHODS

The study was conducted in the Tarleton State University Meat Laboratory. This abattoir is a Texas State Department of Health inspected meat facility. Current Hazard Analysis and Critical Control Point procedures were in place at all times. The temperatures of the coolers used to age the products were monitored and logged continuously (hourly and daily) by the Tarleton State University Facilities Control Center. Monitoring prevented any fluctuation in the temperatures of the cooler being utilized as well as monitoring the defrost cycles of the units.

Product Selection. Twelve paired pork full loins (IMPS #410) as defined by the Institutional Meat Purchasing Specifications (IMPS) ($n = 24$) were obtained from a commercial pork slaughter and meat distribution facility. At 48 hours postmortem, carcasses were tracked during fabrication into bone-in full loins (IMPS #410) and vacuum packaged. Seventy-two hours postmortem the pork loins were shipped to and received by Tarleton State University Meat Science Laboratory. Pork loins were immediately identified and randomly selected for treatment groups. Product was selected from pork carcasses ranging in weights from 55 kg to 80 kg. Bone-in pork loins (IMPS

#410) were randomly assigned to one of two aging temperatures (1.1 °C and 3.3 °C) and five aging times (0, 3, 6, 9, and 12d) for each pork chop from the corresponding pork loins.

Loin Fabrication. Seventy-two hours post-mortem bone-in pork loins were removed from their vacuum packages and blotted dry with paper towels. Again, loins were randomly assigned to one of two aging temperatures (1.1 °C and 3.3 °C) and then fabricated. Pork loins were cut perpendicular to the thoracic bones 5 cm posterior to the blade end using a band saw (Biro, Model #3334; The Biro® MFG Co., OH)

Bone-in center loin chops (2.54 cm thick) were cut and scraped free of bone residue. Chops were further randomly assigned within each temperature (Group A: 1.1 °C and Group B: 3.3 °C) and to one of five storage days (0, 3, 6, 9, and 12d) for aging. Aging periods began following the fabrication of the pork bone-in full loins into pork loin chops (72 hours postmortem). Chops were identified by temperature and storage period, vacuum packaged individually in a 3 mil nylon/polyethylene construction bags (WinPAK, Midwestern Research and Supply Inc., KS) using a table top vacuum packager (Multivac, Model #C200; Multivac Inc., Kansas City, MO).

Aging Treatments. Storage temperatures were monitored (Johnson Control System) using a data logging software by the Tarleton State University Control Center. Temperatures were recorded hourly with defrost periods occurring four times each day (0800, 1200, 1700, and 2400). Pork chops were assigned to and stored at 1.1 °C and 3.3 °C for one of the following periods: 0, 3, 6, 9, or 12 days post fabrication and packaging. The aging times used in this study were selected to represent short, intermediate, and long storage times likely to be found in commercial practice. The short aging time was selected to be well within aging times observed at a retail market.

Warner-Bratzler Shear Force Measurements. An industry standard Warner-Bratzler shear machine made by G-R Manufacturing Company in Manhattan, KS, was utilized in all mechanical tenderness tests. Warner-Bratzler testing and sensory panelists were trained and selected according to the recommended protocol (AMSA 1995).

At the conclusion of the aging periods, the pork loin chops were frozen at -20 °C until instrumental tenderness was measured. Prior to cooking, pork loin chops were thawed for 24 hours at 4.44 °C. Chops were removed from their vacuum packages, blotted dry, and weighed. Weights were recorded pre- and post-cooking (Accu-Weigh, Model #SPC-5005, Yamato). Chops were grilled on an open-face electric pre-heated griddle to 176 °C and to an internal temperature of 70 °C. The internal chop temperatures were monitored during the cooking process using a thermocouple inserted into the geometric center of each chop and recorded using a digital thermometer (Econo Temp™ 32311-K, Cooper-Atkins Corp., CT), (AMSA 1995). During cooking, each chop was turned every four minutes until the desired internal temperature had been achieved. Chops were placed in a cooler at 2 °C and allowed to chill for a 24-hour period until internal temperatures reached between 2 °C and 5 °C. After chilling for 24 hours, a coring device was used to remove three cores from each chop to be used for the Warner-Bratzler shear test. The medial, lateral, and dorsal section cores were sheared perpendicular to the long axis of the core with a Warner-Bratzler shear force unit. Shearing of each core was performed by a V-shaped cutting blade with a triangular aperture of 60° at a velocity of 200 mm/min. Maximum force values were obtained to

generate an average value per sample, indicating the maximum mechanical tenderness value. Peak shear force values were recorded and averaged for each chop.

Microbial Sampling. All microbiological products used within the study were purchased through 3M™ Microbiology, St. Paul, MN, so as to guarantee consistency. Sample collections and platings were performed according to 3M™ Microbiology Petrifilm™ recommendations. The same technician conducted all microbiological testing and recorded all results. This provided consistency and uniformity in the quality of the data collected.

Microbial sampling was conducted on one pork chop from each aging treatment (0, 3, 6, 9, and 12 days post fabrication) prior to freezing at -20 °C and from each temperature treatment group. Microbial sampling provided the data for the study of the shelf-life of temperature controlled wet aged center pork loins. Microbial sampling was conducted using a USDA-FSIS sampling kit for meat and poultry (3M™ Microbiology, St. Paul, MN). Pork chops were sampled by swabbing a sterile sponge across both sides of the chop. Upon completion of the sample collection, the samples were pumped with a stomacher for 30 seconds at 230 RPM (Stomacher® 400 Circulator, Seward). Plating was performed by lifting the top film and holding the pipette perpendicular to the surface. The researcher dispensed 1 mL of the obtained sample suspension into the center of the bottom film. The samples were analyzed using the total aerobic plate count method (APC). This was performed using standard serial dilutions and plate counting methods. Tenfold serial dilutions prepared in 0.1% peptone water were plated on aerobic petrifilms (3M™). A vortex (VWR Analog Vortex Mixer, Henry Troemner LLC.) was used to homogenize dilutions. At a slow rate, the lab technician rolled the top film down on the plate to prevent air bubbles. A plastic spreader was used on the plate with the flat side down in the center of the plate. The lab technician pressed gently on the center of the spreader to distribute the sample evenly on the aerobic plate. The spreader was removed from the surface of the plate and the plate was left undisturbed for at least one minute to allow the sample to form. Plates were incubated in stacks of no more than two plates (Incubator, Model #10-140, Quincy Lab Inc., IL) for 24 hours at 35 °C in a horizontal position with the clear side up. Petrifilms containing between 30 and 300 colony forming units (CFU) (or the highest number if below 30) were enumerated and converted into log CFU to accommodate the anticipated wide fluctuation common to biological data collection.

Initial microbial samples were also plated on Eosin Methylene Blue, MacConkey's agar and Salmonella shigella plates to determine the presence of generic *Salmonella* and *Escherichia coli*. Samples were plated by placing the agars on a flat surface and holding the pipette perpendicular to the agar, dispensing 1 mL of the obtained sample suspension onto the center of the agar. A glass "L" rod was used to spread the sample over the surface of the agar. The "L" rod was submerged in ethanol and flamed to prevent cross contamination between samples. The plates were allowed to aerobically dry and then placed in an incubator held at 35 °C for 24 to 48 hours. The Eosin Methylene Blue, MacConkey's agar and Salmonella shigella plates were then visually analyzed and colony forming units counted for the presence of *Salmonella* and *E. coli* by a trained lab technician.

Sensory Panel. In this study, trained panelists rated the effects of temperatures and aging times on the palatability attributes of the pork loin chops. Sensory panelists were trained

and selected according to the recommendations of the Research Guidelines for Cookery, Sensory Evaluation, and Instrumental Tenderness Measurements of Fresh Meat (AMSA 1995). The same six members of the trained sensory taste panel were utilized throughout the entire study to ensure that the data collected from the sensory panel was consistent.

Panelists were trained to identify quality characteristics of each sample. Prior to conducting sensory analyses, panelists were introduced to a variety of products that varied in tenderness and flavor ratings. Panelists rated each sample on a scale from one to eight, and each sensory characteristic was rated on a scale. The initial and sustained juiciness scale was: 1 = Extremely dry, 2 = Very dry, 3 = Moderately dry, 4 = Slightly dry, 5 = Slightly juicy, 6 = Moderately juicy, 7 = Very juicy, and 8 = Extremely juicy. The scale evaluating initial and overall tenderness was: 1 = Extremely tough, 2 = Very tough, 3 = Moderately tough, 4 = Slightly tough, 5 = Slightly tender, 6 = Moderately tender, 7 = Very tender, and 8 = Extremely tender. The amount of connective tissue was evaluated on the following scale: 1 = Abundant, 2 = Moderately abundant, 3 = Slightly abundant, 4 = Moderate, 5 = Slight, 6 = Traces, 7 = Practically none, and 8 = None. The scale for flavor intensity was: 1 = Extremely bland, 2 = Very bland, 3 = Moderately bland, 4 = Slightly bland, 5 = Slight intense, 6 = Moderately intense, 7 = Very intense, and 8 = Extremely intense. Finally, overall palatability was evaluated on the following scale: 1 = Extremely unsatisfied, 2 = Very unsatisfied, 3 = Moderately unsatisfied, 4 = Slightly unsatisfied, 5 = Slightly satisfied, 6 = Moderately satisfied, 7 = Very satisfied, and 8 = Extremely satisfied. Samples from both aging temperature groups (1.1 °C and 3.3 °C) were equally represented in the samples evaluated for sensory characteristics. Pork chops for sensory analysis were cooked using procedures described for Warner-Bratzler Shear Force. Each chop was trimmed of external fat and bone and fabricated consistent in size, 1.27cm X 1.27 cm and served at consistent internal temperatures of 71 °C. Six panel members for each session participated and samples were served two to five minutes following the cooking process. A total of 120 samples were evaluated by these six trained sensory panelists in this study. Samples were identified by loin, aging period, and temperature group. Each 2.54 cm X 2.54 cm sample was served with unsalted saltine crackers and distilled water to cleanse the palate of the panelists after each sample. Each panelist was instructed to chew each sample 10 times with their incisors and to dispose of the sample upon mastication.

Statistical Analysis. In order to obtain statistical results that were valid, repeatable, accurate, and true representatives of the population, statistical analysis was conducted using the Statistical Analysis System (Barr and Goodnight 1972). For sensory, shear force and microbial data procedure GLM in Statistical Analysis System was used. Model terms included storage temperature and aging length (in days). Least square means were estimated using the LSMEANS statement. When significant effects were detected ($P < 0.05$), the PDIF option was used for mean separation.

RESULTS AND DISCUSSION

Fresh meat is aged to enhance the overall palatability of the product. Aging meat increases tenderness over time, as well as the development of unique sensory characteristics (Dransfield 1994). Therefore, evaluating the effectiveness of temperature impacts on the wet aging of pork loin chops can ultimately lead to improvements in

sensory characteristics and more uniform products for facilitating the marketing of pork products.

Cook loss. In this study, cook loss was not significantly affected by ($P > 0.05$) temperature treatment. Chops stored at 1.1 °C possessed a mean value of 16.69% cook loss and those chops stored at 3.3 °C had a mean value of 17.79% cook loss. The mean values for percent cook loss for the different aging periods were also shown to be insignificant ($P > 0.05$). The most cook loss was observed for the chops aged for nine days (18.70% cook loss). The explanation of the variances in cook loss per day of age is not clearly obvious.

Warner-Bratzler Shear Force. Tenderness of pork has been found to be one of the most important quality factors for consumers. Results suggest that storage temperatures did not affect the Warner-Bratzler values ($P > 0.05$).

However, Warner-Bratzler shear force values decreased as the days of the aging periods increased, (Figure 1, $P < 0.05$). Day 0 was significantly less tender than day 3 of aging.

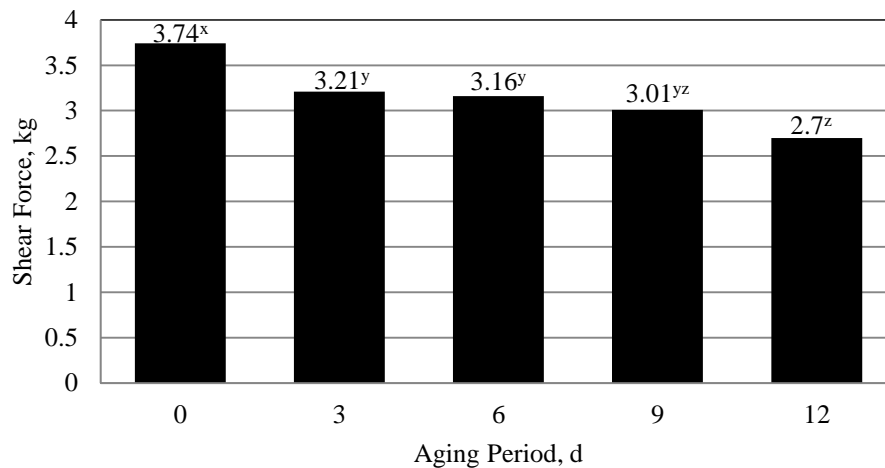


Figure 1. Effect of aging period on Warner-Bratzler shear force values of pork loin chops. ^{x,y,z} Within the figure, means with same superscript are not different ($P < 0.05$).

Between days 3, 6, and 9 of aging, there were no differences between the Warner-Bratzler shear force values ($P > 0.05$). A significant decrease between day six and 12 of aging ($P < 0.05$) is represented in Figure 1, yet no differences were noted for day 9 versus day 12 of aging. Calkins and Seidman (1988) aged beef over a 14-day period and reported a 41.6% change in tenderness from days 3 to 6, 4.0% change in tenderness from days 6 to 12, and a 14.9% change from days 12 to 14. Data reported by Calkins and Seidman (1988) was similar to the results of this study. Data from Davis et al., (2004) indicated a reduction in Warner-Bratzler Shear Force values when the pork loins aged 1 day of age (2.61 kg), 7 days of age (2.26 kg), and 14 days of age (2.19 kg). The results from the Davis study support findings from this study. Results from this study indicate that as chops aged, the shear force mean values decreased.

Microbial Evaluation. Public awareness, concern, and economic impact have increased the number of studies conducted on food-borne pathogens. The increasing research from pork loin chops has led to the developments of more sensitive methods of pathogen prevention, detection, and identification. In this study, initial samples were obtained to determine the presence of generic *Salmonella* and *E. coli*. Samples collected tested negative for both types of bacteria. As represented in Figure 2, total plate counts indicated increasing amounts of bacteria over the various aging periods and higher levels of bacteria in the group stored at 3.3 °C versus 1.1 °C at day 12 only. Findings were supported by Borch et al. (1996), who reported that decreasing refrigeration temperatures subsequently decreased bacterial growth and also affected the shape of the bacteria. Thus, the highest bacteria level was reported at the highest storage temperature and oldest day of aging. Indicated in Figure 2, days 0, 3, and 6 of aging possessed significantly ($P < 0.05$) lower levels of bacteria as compared to days 9 and 12 of aging. Day 12 possessed significantly higher levels of bacteria compared to days 0, 3, 6, and 9 ($P < 0.05$), yet only at the higher storage temperature.

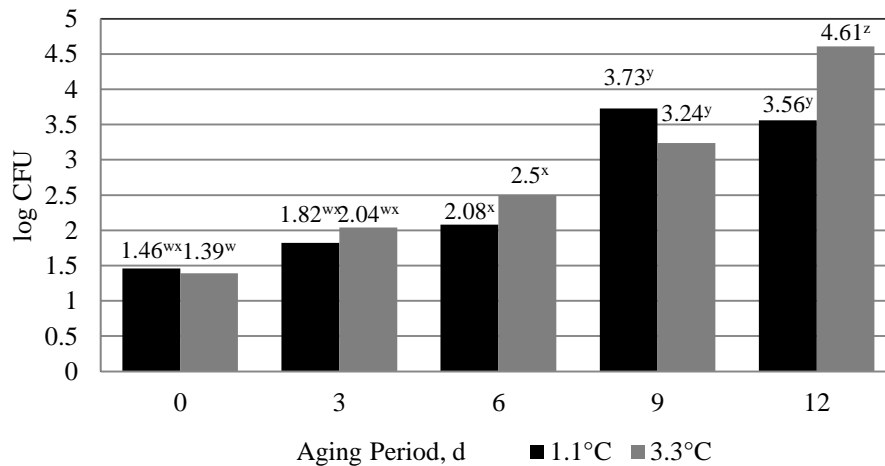


Figure 2. Effects of aging period and total plate counts on shelf-life of pork loin chops.
^{w, x, y, z} Means with uncommon superscript differ ($P < 0.05$).

However, bacteria levels reported by Boers et al. (1994) concluded that if stored properly the pork products do not spoil at a microbial level until 35 days of age. Results from this study indicate that as the loin chops aged, the bacteria levels increased and the aging temperature had no significant effect on bacteria levels except at day 12 of aging.

Sensory Evaluation. Sensory evaluation continues to demonstrate the value and uniqueness of the information it obtains and clearly differentiates it from other product sources (Sidel and Stone 1993). Samples from both of the aging temperature groups were represented in the samples evaluated by the trained sensory panelists. In this study, initial juiciness, sustained juiciness, initial tenderness, overall tenderness, amount of connective tissue, flavor intensity, and overall palatability were evaluated on an eight point scale. Statistics revealed that temperature storage did not significantly ($P > 0.05$) affect any of these palatability characteristics.

Initial Juiciness and Sustained Juiciness. Juiciness of a product is determined by the amounts of water and lipids remaining in muscle after product have been cooked. As stated previously, results from this study suggested that temperature treatment did not affect initial juiciness ($P > 0.05$). However, aging periods significantly affected the initial juiciness and sustained juiciness, respectively, as shown in Figure 3. In Figure 3, day 0 was significantly less juicy than days 3, 9, and 12 of aging. Days 0 and 6 exhibited no difference in initial juiciness ($P > 0.05$).

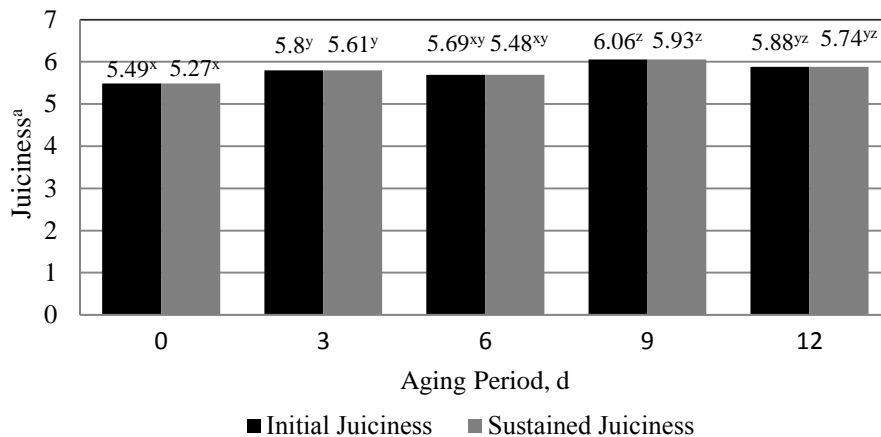


Figure 3. Effect of aging period on initial and sustained juiciness of pork loin chops.

^{x,y,z} Within the figure, means with same superscript are not significantly different ($P < 0.05$).

^a Defines the scale used to evaluate the sample: (1 = Extremely dry, 2 = Very dry, 3 = Moderately dry, 4 = Slightly dry, 5 = Slightly juicy, 6 = Moderately juicy, 7 = Very juicy, and 8 = Extremely juicy).

Day 9 was significantly juicier than days 0, 3, and 6 of aging. Sustained juiciness represented indicated that day 0 was significantly less juicy than days 3, 9, and 12. Similar to initial juiciness, day 0 and 6 showed no difference in sustained juiciness values ($P > 0.05$). Over the various aging periods, day 9 was significantly juicier than days 0, 3, and 6 days of aging. Initial juiciness and sustained juiciness possessed similar trends in levels of juiciness over the aging periods. As the chops aged the levels of initial and sustained juiciness significantly increased ($P < 0.05$). According to Hansen et al. (2004), for pork loin chops aged 0, 4, and 7 days postmortem, there was an overall increase in juiciness occurring as the days of age increased.

Initial Tenderness and Overall Tenderness. Sensory characteristics of initial tenderness and overall tenderness were not significantly affected by temperature ($P > 0.05$) as indicated in Figure 4.

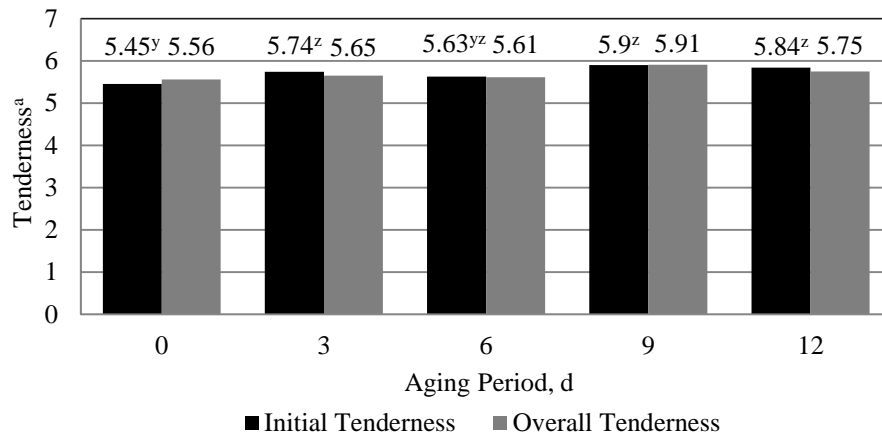


Figure 4. Effect of aging period on initial and overall tenderness of pork loin chops.
^{y,z} Within the figure, means with same superscript are not significantly different ($P < 0.05$).
 Columns lacking a common superscript significantly differ ($P < 0.05$).

^a Defines the scale used to evaluate the sample: (1 = Extremely tough, 2 = Very tough, 3 = Moderately tough, 4 = Slightly tough, 5 = Slightly tender, 6 = Moderately tender, 7 = Very tender, and 8 = Extremely tender)

These findings agree with those of Pierson and Fox (1976), who concluded that ultimate tenderness was not related to the aging temperature of beef longissimus muscle. In Figure 4, day 0 was less tender than days 3, 9, and 12 ($P < 0.05$). There are no differences between days 3, 6, 9, and 12 in initial tenderness values. According to the trained sensory panelists overall tenderness values were not different across all treatment groups ($P > 0.05$). In contrast, Pierson and Fox (1976) reported that tenderness increased as length of aging increased in beef longissimus muscle.

Temperature showed no effects on the amount of connective tissues as noted by the trained sensory panelists. Also, aging effects did not show differences for connective tissue values ($P > 0.05$) based on the sensory panelists. According to Jeremiah and Gibson (2003), as beef is held postmortem the amount of perceptible connective tissue decreased as postmortem aging was prolonged. Contrary to Jeremiah and Gibson (2003), Pierson and Fox (1976) stated that the length of aging time and temperature had no effect on the amount of connective tissue in a sample.

Flavor Intensity. Flavor intensity was not affected by temperature. In Figure 5, day 0 of aging the flavor intensity possessed lower flavor intensity as compared to days 3, 9, and 12 ($P < 0.05$).

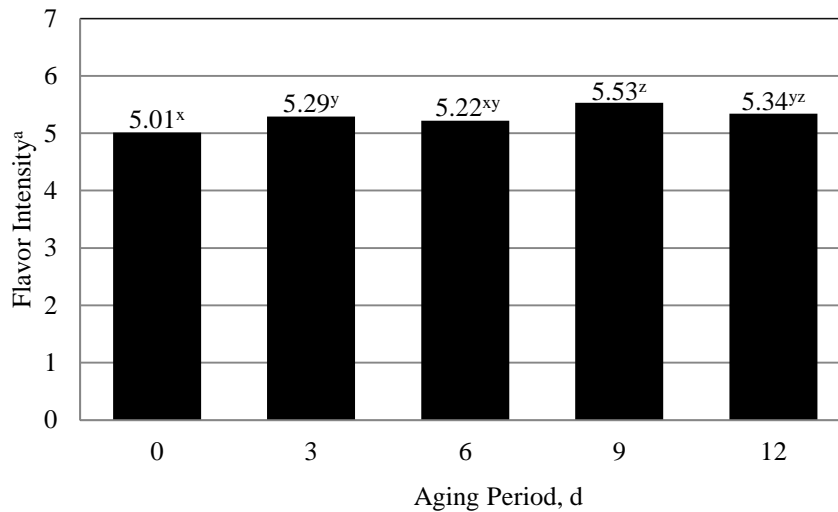


Figure 5. Effect of aging period on flavor intensity of pork loin chops.

^{x,y,z} Within the figure, means with same superscript are not significantly different ($P < 0.05$).

^a Defines the scale used to evaluate the sample: (1 = Extremely bland, 2 = Very bland, 3 = Moderately bland, 4 = Slightly bland, 5 = Slight intense, 6 = Moderately intense, 7 = Very intense, and 8 = Extremely intense).

In consideration of days 3, 6, and 12, there were no significant changes in flavor intensity ($P > 0.05$). Day 9 possessed the most intense flavor ($P < 0.05$), yet showed no difference when compared to day 12. Flavor intensity was affected by aging but there was not a clear explanation or pattern explaining the significant changes in flavor intensity. According to data from Hendrix et al. (1963), 1,541 members of a consumer panel criticized pork for having an unsatisfactory flavor. Chops with higher percentages of expressible juices and lipids provided the most intense flavor (Davis et al. 1975). These findings disagree with the slightly intense flavor profile that the trained sensory panelists noted in this study.

Temperature did not significantly affect overall palatability. Furthermore, days of aging did not significantly affect overall palatability of the loin chops as detected by the sensory panel. Jennings et al. (1978) found similar results indicating that there was no substantial improvement in overall palatability in beef to warrant vacuum packaged storage for those cuts that are more than 10 days postmortem. In contrast of Jennings et al. (1978), Jeremiah and Gibson (2003) indicated beef wet-aged past 28 days possessed unique palatability characteristics.

CONCLUSION

Product tenderness continues to be of concern in the meat industry. Results from this study may assist retail and foodservice operators in establishing appropriate postmortem aging times for bone-in pork loin chops. Clearly, pork tenderness is a complex sensory attribute influenced by many factors. The basis of pork tenderness and tenderization must be understood before this quality characteristic can be improved. In

this study, the effectiveness of temperature controlled pork wet aging and its relationship to tenderness, juiciness, and shelf life of pork loins was evaluated.

Results from this study indicated that neither temperature nor aging period had any effect on the cook loss of loin chops. Furthermore, temperature did not affect the Warner-Bratzler shear force values; however, varying aging periods had significant effects on the Warner-Bratzler values. As the loin chops aged, the Warner-Bratzler values significantly decreased therefore, increasing the level of tenderness in the product. By increasing the length of times that pork loin chops are wet-aged, the more tender the product will become.

In addition, this study proved that as the length of time the loin chops were aged the microbial levels significantly increased (APC), yet not to dangerous levels that would support food-borne illnesses. The two temperature storage levels did not have an effect on the microbial levels. Sensory results showed that initial juiciness, initial tenderness, and flavor increased with aging of pork loin chops, yet no differences were reported for overall tenderness and palatability. Understanding the process of aging can result in providing the pork industry with new methods of creating a more uniform more tender product while maintaining proper levels of food safety and meeting all sensory levels required by the consumer.

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Effects of Antioxidant Application and Retail Display on Sensory, Shelf Life, and Oxidative Stability of Beef Striploin Steaks¹

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ABSTRACT

Beef strip-loin steaks were treated with GRAS (generally recognized as safe) approved antioxidant formulations: citric acid (CIT) 0.3%, synthetic butylated hydroxyanisole/butylated hydroxytoluene (SYN) 0.08% and a control (CON) of distilled water. Strip-loins (IMPS # 180) (n = 32) at postmortem day 14, 21, and 28 were removed from vacuum packages and fabricated into 2.54-cm steaks. Steaks (n = 63) were randomly assigned to treatment groups (n = 21 samples/treatment). After treatment steaks were over-wrapped, and placed in simulated retail display for five display days (DD). Sensory attributes were not affected by treatment ($P > 0.05$). SYN treatment maintained strip-loin color ($P < 0.05$) while CON and CIT exhibited additional discoloration in DD 4 and 5 compared to SYN across ageing periods. CON and CIT contained additional browning in DD 3-5 ($P < 0.05$) than SYN. SYN exhibited greater a^* values through DD periods ($P < 0.05$) when compared to CON or CIT. SYN exhibited greater oxymyoglobin level ($P = 0.03$) and reduced metmyoglobin levels ($P < 0.05$) throughout DD and PM ageing. CON and CIT exhibited increased oxidation on DD 1, 3, and 5 compared to SYN ($P < 0.05$). SYN application prolonged retail display shelf life by maintaining color and reducing lipid oxidation without negatively affecting sensory attributes.

KEY WORDS: beef, striploin, antioxidant, shelf life, sensory

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INTRODUCTION

Overall, the meat industry's role is to provide quality products to satisfy needs of the consumer in the world (Resurreccion 2003). Depending on the region, over one-fourth to one-third of the worldwide production of meat is lost every year because of deteriorating product quality (Oussalah et al. 2004). It has been estimated that average value deterioration is 3.7% for the entire meat department and 5.4% for fresh meat (Williams et al. 1992). Williams et al. (1992) also stated that the U.S. industry stands to gain \$175 million to \$1 billion annually by increasing case life by one to two days. A percentage of this magnitude gives prime evidence why more research and development is needed for the preservation and maintenance of fresh retail meat products. Changing consumer demands in the U.S. have influenced the market for all types of meat. Due to these overall changes in demands, the meat products that are produced for sale and consumption must be of high standard quality and transportable to vast marketplaces located long distances away from production facilities. Meat is a highly perishable food; both oxidative and microbial processes are involved in meat spoilage (Camo et al. 2008). Flavor, color and shelf life all are related to the oxidative state of meat. A major setback cited by supermarket meat managers is discoloration which cause beef retail products to be discounted or discarded depending on expiration date or shelf life of the product (Steiner et al. 2001). When muscle cuts are discolored on or before the sell by date, retail cuts must be marked down in price, faced, or repackaged (Smith et al. 1993).

When products are re-handled or the form of original product is changed, there is usually a loss of quality and value, along with the added chance of microbial contamination. The average consumer bases the purchase of a product predominantly on visual appearance, of which color is the number one factor (Marth 1998). A bright cherry red color characterizes a freshly cut steak and is the desired visual characteristic of choice. Meat markets, which still use over wrap packaging of products, have an increased problem with oxidation and bacterial loads when compared to products packaged in a modified oxygen environment (Jeremiah and Gibson 1997). Oxidation is a chemical change of fat and muscle pigments that leads to rancidity, color changes and flavor deterioration. Over wrap is the most economically feasible method for retail display, but there is limited control of oxygen levels and other environmental factors associated with product deterioration. Lipid and protein oxidation is a critical point for red meat packaged under aerobic conditions, since it occurs at the same rate as discoloration and faster than microbial growth (Camo et al. 2008). Past research has focused on the use of antioxidants in ground and processed meat products for oxidative stability and overall shelf life (Greene et al. 1971). Limited research has been conducted on the basis that defines effects of antioxidant application on color, oxidative stability, and shelf life of whole muscle beef steaks over extended time in a retail environment. Correspondingly, the objectives of the current study are to evaluate: 1) effects of specified antioxidants on sensory properties of beef strip-loin steaks, and 2) effects of specified antioxidants on surface color and oxidative stability of fresh beef striploin steaks.

MATERIALS AND METHODS

Treatments. Experimental treatments consist of two antioxidant formulations and a control to determine the overall effect of antioxidant application. Control group (CON) was applied with distilled deionized water. Antioxidant treatments consisted of 0.3%

citric acid solution (CIT; 1 L distilled deionized water + 3 g of food grade citric acid) and 0.08% solution of butylated hydroxyanisole and butylated hydroxytoluene (SYN; 1 L distilled deionized water + 0.4 g Butylated hydroxyanisole and 0.4 g butylated hydroxytoluene) (Table 1; Integra Chemicals, Kent, WA). Solution preparation consisted of heating distilled deionized water to 37 °C, and then adding chemical component into water under high shear. These formulations were calculated once the level of uptake was determined to be 4 g solution / striploin steak sample. All antioxidant treatments were formulated in accordance to guidelines set forth in CFR 184.0(b)(1).

Table 1. Experimental treatment formulations applied to striploin steaks.

Treatment ^a	Formulation
Control (CON)	Distilled deionized water
Citric acid (CIT)	^b 0.3% citric acid + distilled deionized water
Butylated hydroxyanisole/ Butylated hydroxytoluene (SYN)	^c 0.08% Solution (BHA/ BHT) + distilled water

^a All treatments resulted in a uptake equaling 4.0 g of solution formulated based on accordance guidelines set forth in CFR 184.0(b)(1).

^b Citric acid treatment (0.3% solution) 3 g citric acid/ 1 L distilled deionized water.

^c Butylated hydroxyanisole/ Butylated hydroxytoluene treatment (0.08% solution) 0.4 g BHA 0.4 g BHT/ 1 L distilled deionized water.

An automated belt line was utilized to transport each sample through an atomization cabinet where application of treatment solution (CON, CIT, and SYN) from a top mounted surface nozzle was applied while a constant level of pressure (275,790 pa) was maintained. A constant speed setting was used on the belt line to increase the rate of accuracy of treatment uptake of 4 g per steak. Sample weight was taken before and after application of treatment to ensure application of 4 g of solution per steak.

Sample Selection. Wholesale beef striploins (n = 32; IMPS #180, USDA, 1996) were obtained from a commercial beef plant in Plainview, TX, vacuum packaged, and transported to the Angelo State University Food Safety and Product Development Laboratory under refrigerated storage. Temperature recorders (DataWatch, Global Sensors, Belmont, NC) were utilized to ensure striploin temperatures remained below 4 °C during transport. Wholesale striploins were selected within a specified window of USDA quality and yield grading to minimize inherent variation. All selection criteria were evaluated by trained personnel according to USDA quality and yield grade guidelines (USDA 1989).

Sample Preparation. Striploins were wet aged in vacuum package bags for 14, 21, and 28 days postmortem to simulate the approximate time beef remains in storage and transit prior to consumer availability. Sixteen wholesale strip-loins were utilized per trial. On days 14, 21, and 28 postmortem strip-loins were fabricated into four 2.54 cm thick steaks cut from the anterior end of each strip-loin. One steak of the total was randomly discarded to achieve equal representation within each of three treatments. Steaks (n = 63) were then randomly assigned to one of the three treatment groups (CON, CIT, or SYN) to achieve n = 21 samples/treatment. Strip-loin steaks within each treatment were then trimmed down to 0.06 cm external fat thickness to maintain sample consistency. Within each treatment group (n = 21) steaks were randomly assigned to one of three display day

(DD) ageing treatments (DD 1, DD 3, and DD 5) to achieve seven steaks per DD ageing treatment. Within each DD ageing treatment, four steaks were assigned to lipid oxidative stability analysis. Three steaks were assigned to sensory analysis within each DD ageing treatment combination. Steaks for lipid oxidative stability and sensory evaluation analysis were removed from simulated retail display according to specified DD ageing treatment, vacuum packaged and stored at -0 °C for subsequent analysis.

Simulated Retail Display. Immediately post treatment application steaks were placed on a standard retail grade Styrofoam tray, coded with sample identification and overwrapped with polyvinyl chloride film (PVC) to mimic retail presentation. Within one hour of treatment application, all steaks were placed in simulated retail display. Steaks were randomly placed in a Tyler retail display case (Model NM8, Tyler Refrigeration Corporation, Niles, MI) to mimic retail display conditions. Case temperature was maintained at 4 °C and exposed to optimum retail display lighting (Promolux Safe Spectrum T8 Platinum, Shawnigan Lake, BC, Canada) with illumination intensity maintained near 1900 lux. Simulated retail display was adapted from a previously published procedure (Braden et al. 2007).

Sensory Evaluation. Prior to the start of evaluation, a sensory training session was conducted in reference to procedures of Cross et al. (1978) so trainees would understand testing procedures and evaluation of sample meat products. Steaks were removed from frozen storage and placed under refrigeration (4 °C) 24 hours prior to sensory evaluation. Steaks were cooked on a George Forman electric clam shell style grill (Applia Consumer Products, Bedford Heights, OH) to an internal temperature of approximately 71 °C to achieve a medium degree of doneness according to procedures outlined by Kerth et al. (2003). Steaks were then cut into 1-cm³ cubes and stored in warming pans until the entire panel was prepared. All samples were served to sensory panel warm within 15 minutes of cook time. At least six trained individuals were utilized for sensory evaluation during each panel. Apple juice, unsalted crackers, and water were provided to each panelist to properly cleanse the pallet between each sample. Steaks were analyzed for initial and sustained juiciness, initial and sustained tenderness, flavor intensity, off flavor, and overall acceptability according to ballot scaling procedures of Cross et al. (1978).

Visual Color Evaluation. Subjective color training was conducted prior to research to ensure no color vision deficiencies were present in the panel. During a five-day display period, steaks were evaluated daily by a trained panel, consisting of at least six members. The daily color evaluation was conducted within a one hour window for daily evaluation throughout each postmortem period for beef color, color uniformity, surface discoloration, and lean browning according to AMSA (1991) color panel evaluation guidelines.

Objective Color Evaluation. Commission Internationale de l'Eclairage (CIE) L* (muscle lightness), a* (muscle redness), b* (muscle yellowness), and reflectance spectra values were determined daily through the overwrap for display day from two random readings on each steak with a Hunter Miniscan XE Plus (Hunter Associates Laboratory, Inc., Reston, VA) using illuminate D65 at 10° and a 3.5 cm aperture. Spectral reflectance values were determined and recorded every 10 nm over a range of 400-700 nm. Muscle chroma (color intensity/saturation), hue, angle (wavelength of light radiation red, yellow,

green, blue, and purple), myoglobin (fresh muscle pigment), oxymyoglobin (oxygenated muscle pigment), and metmyoglobin (brown oxidized muscle pigment) values were obtained utilizing equations as described by Hunt (1980) and Clydesdale (1991). Visual and instrumental color analysis protocols were utilized similar to those as presented by Braden et al. (2007).

Oxidative Stability Assessment. Lipid oxidative stability was determined utilizing a thiobarbituric acid (TBA) reactive substance assay as detailed by Buege and Aust (1978). Lipid oxidative stability samples from display days 1, 3, and 5 were removed from frozen storage and a 10 g sample was homogenized with 30 mL of distilled water. Approximately 4 mL of homogenate was combined with 8 mL of trichloroacetic/thiobarbituric acid reagent and 100 μ L of 10% butylatedhydroxyanisole. Samples were then incubated in a 99 °C water bath for 15 minutes, allowed to cool in cold water (4 °C) for 10 minutes and spun at 2000 x g for 10 minutes. The absorbance of the supernatant was then read against a blank containing like reagents at 531 nm. Malonaldehyde standard, utilizing 1,1,3,3-tetraethoxypropane and thiobarbituric acid, was used and thiobarbituric acid substances were reported as mg/10g of meat.

Statistical Analysis. Sensory and TBA data was analyzed as a completely randomized design using the general linear models procedures of SAS (SAS Inst. Inc., Cary, NC). Sensory and TBA data was included in the model with treatment as a fixed effect. DD 1, 3, and 5 visual and instrumental color data was analyzed for a completely randomized design, with a split plot repeated measures arrangement using the mixed models procedures as implemented in PROC MIXED (Littell et al. 1996; SAS Inst. Inc., Cary, NC). Visual color, lean uniformity, lean discoloration, lean browning, L*, a*, b*, Chroma, hue, myoglobin, oxymyoglobin, and metmyoglobin values were included in the model with treatment and display day and all two way interactions as fixed effects. Display day was analyzed as a repeated measure with steak as the subject of the repeated statement and based on AICC criteria an optimum covariance structure was selected (Littell et al. 1996). Steak served as experimental unit and significant ($P \leq 0.05$) treatment effect means were separated using Fisher's protected LSD.

RESULTS AND DISCUSSION

Color Measurements. All subjective measurements taken by trained panelists deteriorated over time, as steaks reached the extent of their display day periods throughout each postmortem aging period. Color score was evaluated for treatment x display day x postmortem aging ($P < 0.001$; Figure 1). When controlling for display day, there was no treatment x postmortem aging ($P = 0.51$) effect. A desired bright cherry red color was maintained by SYN treatment for a longer period of time when compared to CON and CIT, especially during display days 3, 4, and 5 of postmortem aging period 28 (Figure 2). For lean discoloration scores there was treatment main effect ($P = 0.01$) and for treatment x display day x postmortem aging ($P < 0.001$; Figure 3); but not for treatment x display day ($P = 0.11$) or treatment x postmortem aging ($P = 0.37$). SYN treatment exhibited less discoloration in later display day periods of postmortem aging periods 21 and 28 when evaluated by panelist. Lean browning was effected by treatment x postmortem aging x display day ($P < 0.001$). A lower level of browning was observed by panelist for steaks from SYN treatment throughout all three postmortem periods.

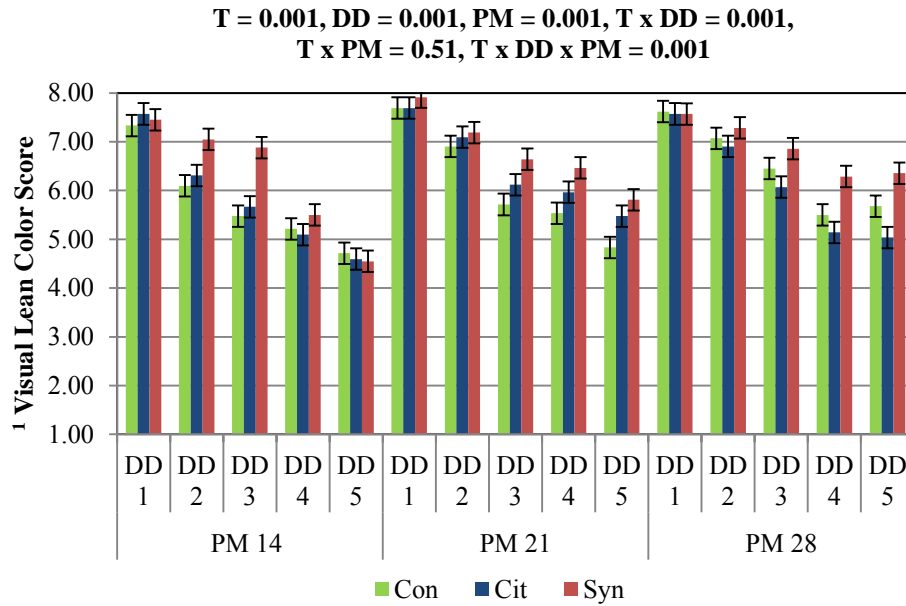


Figure 1. Least square means \pm SEM for visual lean color of striploins by retail display day within postmortem aging days. Treatments consisted of control (CON), citric acid (CIT), and BHA/BHT (SYN); T = treatment, DD = days of simulated retail display, and PM = days of postmortem aging ¹Lean color uniformity (1 = extremely dark red; 8 = extremely bright cherry-red).

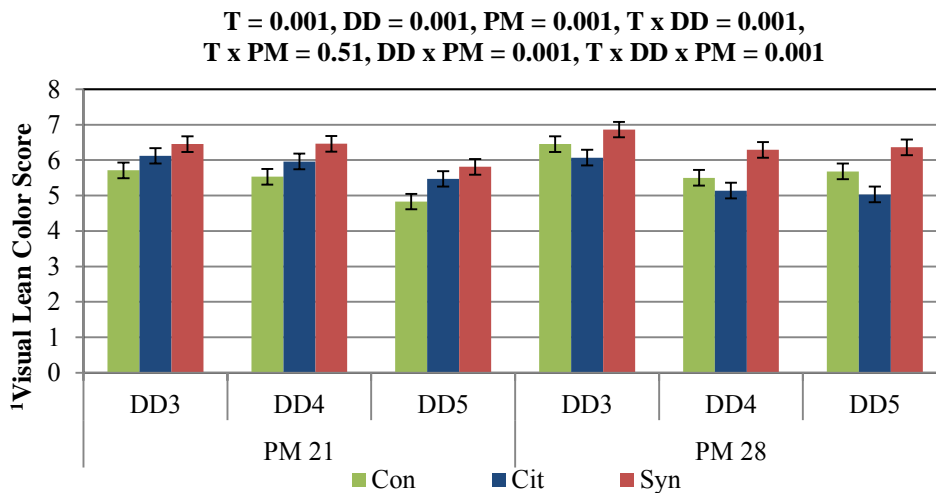


Figure 2. Least square means \pm SEM for visual lean color of striploins by retail display day (3-5) within postmortem aging days (21 and 28). Treatments consisted of control (CON), citric acid (CIT), and BHA/BHT (SYN); T = treatment, DD = days of simulated retail display, and PM = days of postmortem aging ¹Lean color uniformity (1 = extremely dark red; 8 = extremely bright cherry-red).

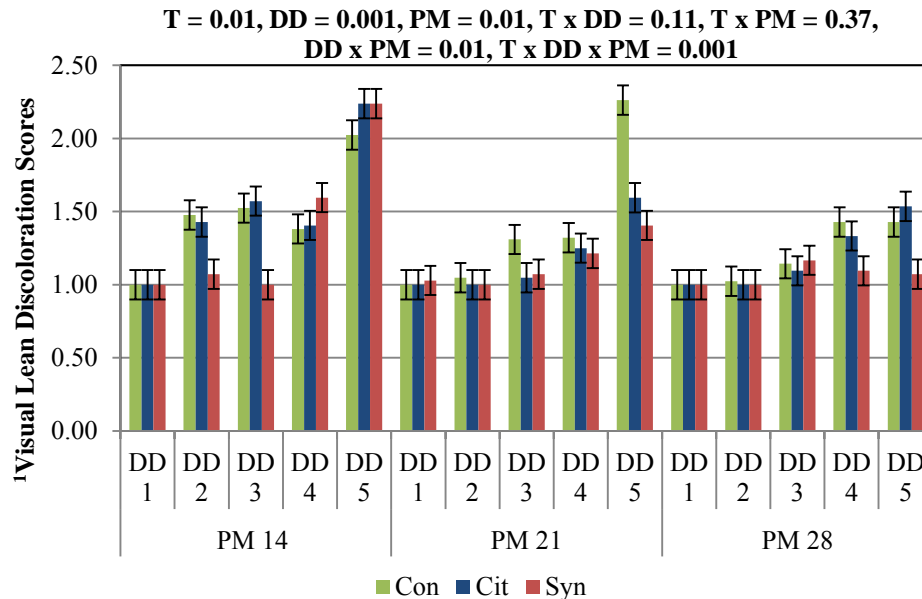


Figure 3. Least square means \pm SEM for visual lean discoloration of striploins by retail display within postmortem aging days. Treatments consisted of control (CON), citric acid (CIT), and BHA/BHT (SYN); T = treatment, DD = days of simulated retail display, and PM = days of postmortem aging; ¹Lean discoloration (1 = 0%; 7 = 100%).

With increased postmortem and retail display day aging, all objective color attributes declined ($P < 0.05$). As these objective color measurements decreased there were varied rates of decline according to variable, treatments, treatments x display day, treatment x postmortem aging, and treatment x display day x postmortem aging combinations.

CIE L* values ($P < 0.001$), were slightly lower for SYN treatments when compared to CON and CIT treatments. No two way interaction for CIE L* values were seen for treatment x display day ($P = 0.74$) or treatment x postmortem aging ($P = 0.13$). There was an interaction for treatment effect on CIE a* values for treatment x postmortem aging ($P = 0.004$; Figure 4) but not for treatment x display day ($P = 0.52$). When comparing a* values between the three treatments during each postmortem period the SYN treatment had higher overall values, being more red when compared to the CON and CIT application. CIE b* values were similar to a* findings as interactions of treatment x postmortem aging ($P < 0.001$; Figure 5) along with treatment x display day ($P < 0.001$) values tended to be higher (more yellow) in steaks receiving SYN treatment. There were no interactions of display day x treatment ($P = 0.76$) or treatment x display day x postmortem aging ($P = 0.87$). Chroma values did show a positive treatment x postmortem aging affect ($P < 0.001$; Figure 6) where SYN treatment maintained higher levels of color saturation when compared to CON and CIT in all ageing periods. There were no interactions of treatment x display day ($P = 0.61$) or treatment x display day x postmortem aging ($P = 0.54$) when evaluating chroma. For Hue, there was only a positive treatment effect ($P < 0.001$) where SYN had lower hue values when compared to CON and CIT treatments. When evaluating myoglobin levels, there were interaction effects

due to treatment x display day x postmortem aging ($P < 0.001$; Figure 7). Myoglobin (fresh muscle pigment) levels were maintained for longer periods of time by SYN treatment in the later of display days 3, 4, and 5. When samples were evaluated for oxymyoglobin content there was an effect due to treatment x postmortem aging x display day ($P = 0.011$; Figure 8). The oxymyoglobin levels were maintained higher for SYN treated steaks when comparing display days 4 and 5 throughout all three postmortem periods. Metmyoglobin levels were substantially dependent upon treatment effect ($P < 0.001$) were SYN (36.38) was lower in value for oxidized myoglobin pigment when compared to CON or CIT (38.52 and 38.28, respectively). No two-way or three-way interactions for treatment had an effect ($P > 0.05$).

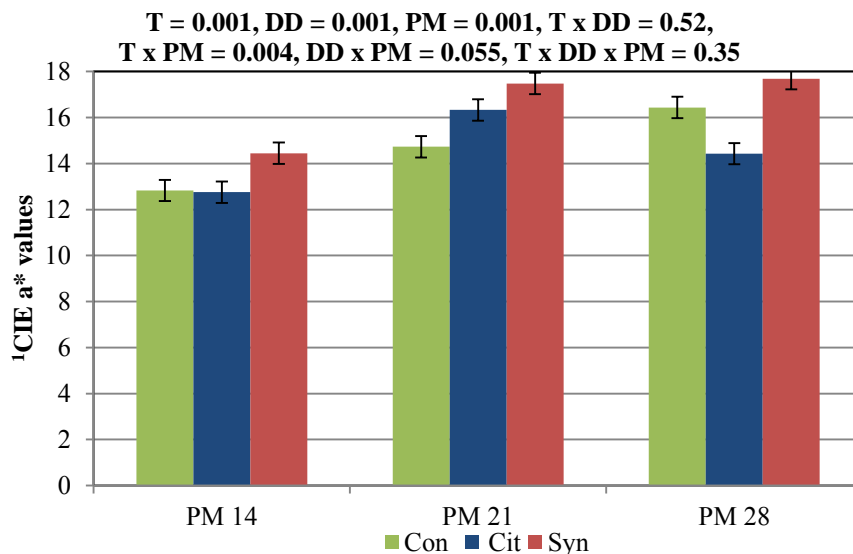


Figure 4. Least square means \pm SEM for CIE a* instrumental values of striploin steaks by postmortem aging period. Treatments consisted of control (CON), citric acid (CIT), and BHA/BHT (SYN); T = treatment, DD = days of simulated retail display, and PM = days of postmortem aging; ¹CIE a* value (positive = red, 0 = neutral, negative = green).

According to Mancini and Hunt (2005), meat color is the greatest quality factor that influences a meat purchasing decision made by consumers since it's an indicator of freshness and wholesomeness. As a result, nearly 15% of retail beef is discounted in price due to surface discoloration, which corresponds to annual revenue losses of \$1 billion (Smith et al. 1993). Visual color analysis provides evidence that is more comparable to consumer's perception of color than instrumental color analysis. The use of instrumental color analysis does provide scientific measurements that are widely accepted and comparable throughout many different criteria. Economic improvements associated with products that improve color life potential and stability has been sought after for many years.

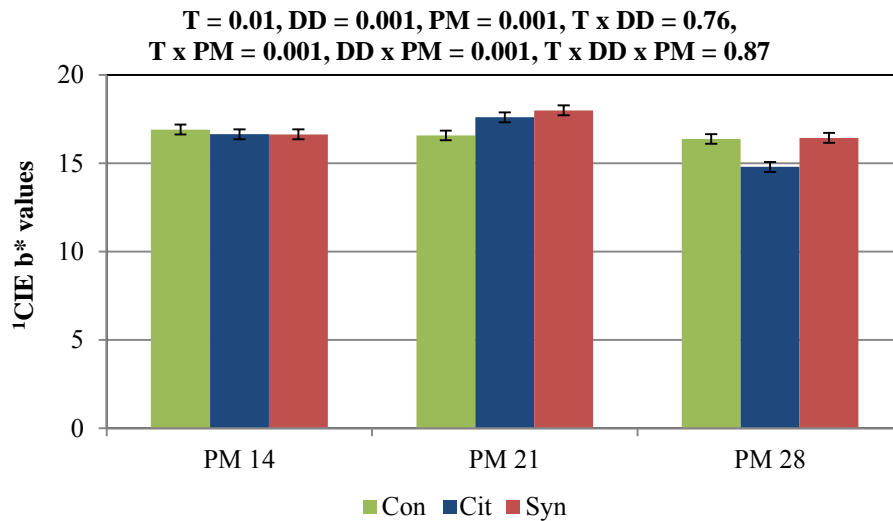


Figure 5. Least square means \pm SEM for CIE b* instrumental values of striploin steaks by postmortem aging days. Treatments consisted of control (CON), citric acid (CIT), and BHA/BHT (SYN); T = treatment, DD = days of simulated retail display, and PM = days of postmortem aging; ¹CIE b* Value (positive = yellow, 0 = neutral, negative = blue).

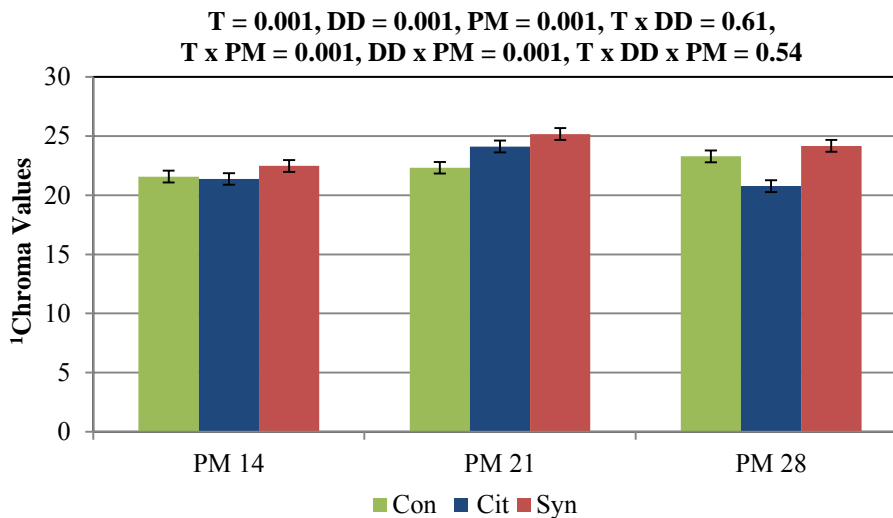


Figure 6. Least square means \pm SEM for lean chroma instrumental values of striploin steaks by postmortem aging days. Treatments consisted of control (CON), citric acid (CIT), and BHA/BHT (SYN); T = treatment, DD = days of simulated retail display, and PM = days of postmortem aging; ¹Lean Chroma Value (numerically increasing color saturation).

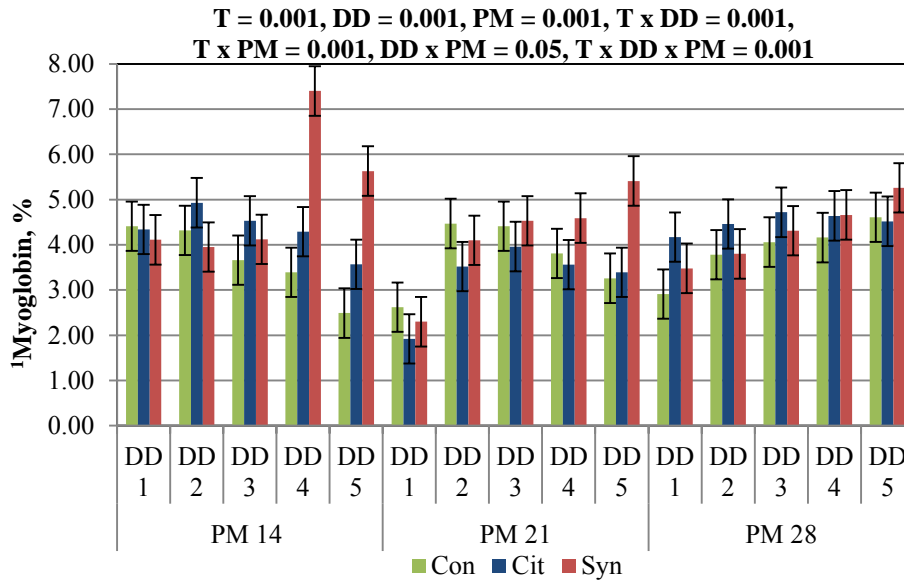


Figure 7. Least square means \pm SEM percent myoglobin instrumental values of striploin steaks by retail display day within postmortem ageing days. Treatments consisted of control (CON), citric acid (CIT), and BHA/BHT (SYN); T = treatment, DD = days of simulated retail display, and PM = days of postmortem aging; ¹Myoglobin percentage (fresh muscle pigment).

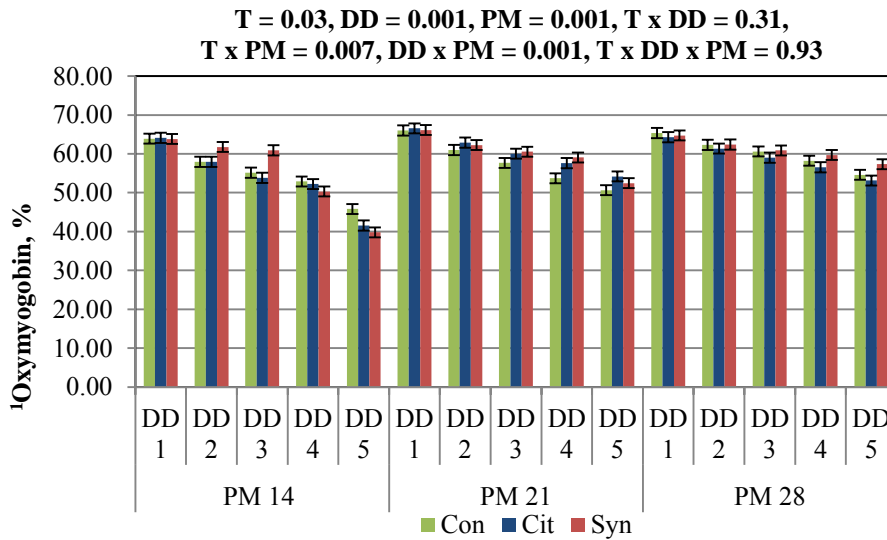


Figure 8. Least square means \pm SEM for lean oxymyoglobin instrumental values of striploin steaks by retail display day within postmortem ageing days. Treatments consisted of control (CON), citric acid (CIT), and BHA/BHT (SYN); T = treatment, DD = days of simulated retail display, and PM = days of postmortem aging; ¹Oxmyoglobin percentage (Oxygenated myoglobin pigment).

The effect of SYN application on striploin steaks was increasingly evident in the advanced periods of display day within all three postmortem periods. CIT application exhibited less of an effect on color stability when compared to SYN treatment. CIT application actually increased discoloration scores and browning scores during subjective color analysis when compared to CON results. The effect of SYN application was visibly noticeable throughout the simulated retail display setting as redness and color uniformity was extended for an additional 1-2 days when compared to CON and CIT treatments. When analyzing each postmortem aging periods from display days 1 through 5 SYN treatment maintained visual color scores while CON and CIT had significant spikes on display days 3 through 5.

The effect of SYN treatment on striploin steaks on instrumental a^* values showed the most significant trend in terms of meat redness as SYN maintained mean values of (16.54) compared to CON (14.66) and CIT (14.51).

Sensory. Sensory characteristics were evaluated on striploin steaks to compare treatment effects (Table 2). No effect of treatment on initial juiciness ($P = 0.815$), or sustained juiciness from treatment ($P = 0.768$) was evident from the panelist. There was also no effect from treatment on initial tenderness ($P = 0.281$) or sustained tenderness ($P = 0.769$). There was no effect of treatment on beef flavor intensity ($P = 0.463$), off flavor ($P = 0.158$) and overall acceptability ($P = 0.597$). Treatments of CON, CIT, and SYN did not produce any detectable differences in relation to the sensory properties of overall striploin steaks.

Several different aspects must be considered when the addition of any chemical compound or additive is made to a meat or food product. It must not influence the flavor profile of the item in any negative way and also must not change the juiciness or tenderness properties. During the cooking process chemical reactions occur between fatty acids and amino acids and their degradation products provide a large number of compounds that can contribute to meat flavor (Wood et al. 2004). Off odors and flavors are one of the most common negative sensory aspects developed during the ageing process of meat products. As with a study conducted by Camo et al. (2008), the direct addition of rosemary extract extended the fresh odor and color from eight to 13 days when compared to the control that contained no extracts. As seen with the results from this study there was no difference for any sensory attributes throughout the five-day display period for all three treatments. As determined by Morrissey et al. (1998), the typical shelf life of fresh meat is usually a time period of 3-5 days. Due to this information, there were no major off flavors produced since each postmortem aging period only had five days in a retail case environment. All sensory characteristics in the current study were similar across treatments. Sebranek et al. (2005) determined no unusual or uncharacteristic flavors were detected by panelist in a study where BHA/BHT was applied to pork sausage to determine its antioxidant effectiveness along with natural rosemary extract. Typical spices utilized in the production of pork sausage could have masked potential off flavors associated with synthetic antioxidants. In the present study, strip-loin steaks were not exposed to any spices or flavoring other than applied treatments of citric acid and BHA/BHT an acceptable determination could be made of sensory characteristics. Due to the very low concentration levels of antioxidants applied, citric acid 0.3% and BHA/BHT solution 0.08%, there were no detectable chemical flavors produced.

Table 2. LS Means \pm SE of Sensory Attributes of Striploin Steaks.

Attribute	Control	Citric Acid	Synthetic Acid
Average Initial Juiciness ^a	5.85 \pm 0.14	5.78 \pm 0.14	5.72 \pm 0.14
Average Sustained Juiciness ^b	5.56 \pm 0.13	5.51 \pm 0.13	5.43 \pm 0.13
Average Initial Tenderness ^c	6.20 \pm 0.24	5.70 \pm 0.24	5.78 \pm 0.24
Average Sustained Tenderness ^d	5.81 \pm 0.14	5.66 \pm 0.14	5.75 \pm 0.14
Average Flavor Intensity ^e	5.88 \pm 0.08	5.83 \pm 0.08	5.75 \pm 0.08
Average Off Flavor ^f	3.78 \pm 0.04	3.75 \pm 0.04	3.86 \pm 0.04
Average Overall Acceptability ^g	5.65 \pm 0.14	5.47 \pm 0.14	5.47 \pm 0.14

^a (Initial Juiciness) 1=Extremely Dry, 8=Extremely Juicy

^b (Sustained Juiciness) 1=Extremely Dry, 8=Extremely Juicy

^c (Initial Tenderness) 1=Extremely Tough, 8=Extremely Tender

^d (Sustained Tenderness) 1=Extremely Tough, 8=Extremely Tender

^e (Flavor Intensity) 1=Extremely Bland, 8=Extremely Intense

^f (Off Flavor) 1=Extreme Off Flavor, 4=None

^g (Overall Acceptability) 1=Dislike Extremely, 8=Like Extremely

Lipid Oxidation. Thiobarbituric reactive substances (TBA) were dependent on treatment x display day ($P = 0.013$; Figure 9). TBA levels increased with extended display day exposure for CON and CIT while SYN treatment maintained almost constant lower TBA values through display days 1 through 5. Since TBA is an indicator of lipid oxidation compounds this provides evidence that SYN treatment was not oxidizing as fast of a rate as other two treatments.

The typical oxidative deterioration of meat and meat products is caused by the degradation reactions of fats and pigments. Many of the oxidative processes that occur in meat can lead to other organoleptic deterioration in taste, color and texture. Antioxidants, including vitamin E which is commonly utilized in feedstuffs is a primary lipid soluble antioxidant in biological systems and breaks the chain of lipid peroxidation in cell membranes and prevents the formation of lipid hydroperoxides (Halliwell 1987). As determined with results from Sebranek et al. (2005), utilizing synthetic antioxidants such as BHA/BHT combinations are effective in maintaining low thiobarbituric reactive substance (TBARS) values of pre-cooked meat products. The effects of selected antioxidants from this study on the oxidative stability of strip-loin steaks in a retail case environment are presented (Figure 9). We found measures of lipid oxidation (TBARS) to increase with extended display day exposure and vacuum-packaged postmortem aging periods. TBARS increased in CON treatments and CIT treatments at an increased rate, when compared to SYN treatments especially in the later display day periods. As the data indicates SYN treatment had a relatively small increase from display day 1 through display day 5. This is a good indication that oxidation rates were reduced to a rate that could possibly increase the number of days before oxidative compounds are formed. Results from Sebranek et al. (2005) determined that BHA/BHT treatments was the most effective method in keeping TBARS at or below the baseline value of 0.5 mg/kg for up to 11 days in pork sausage. Data from the current study on display day 5 SYN has a mean value of 0.11 mg/kg as compared to CON values of 0.32 mg/kg for striploin steaks.

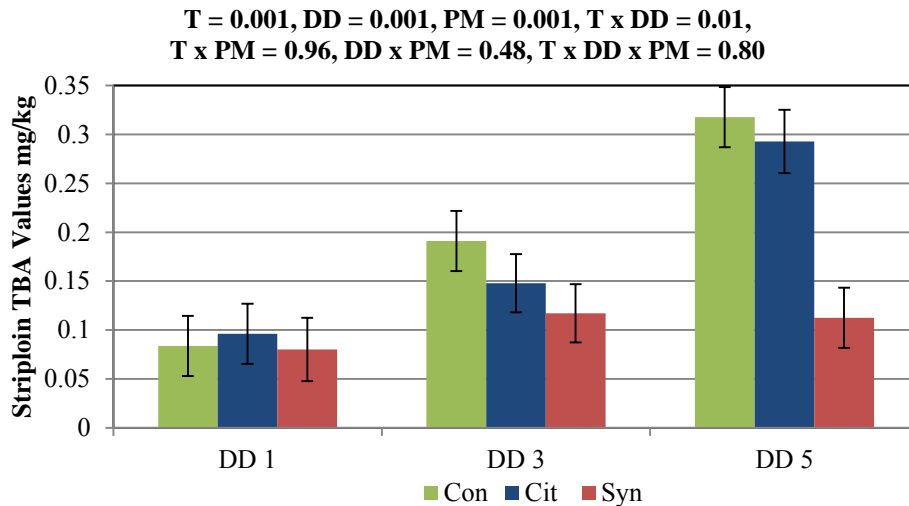


Figure 9. Least square means \pm SEM for thiobarbituric reactive substances values of striploin steaks by retail display day. Treatments consisted of control (CON), citric acid (CIT), and BHA/BHT (SYN); T = treatment, DD = days of simulated retail display, and PM = days of postmortem aging.

Master bag packaging extends the storage of the meat product but does not provide any noticeable benefits once the product is placed on the retail shelf. This is a major aspect in which antioxidant application could be utilized to extend the shelf life of whole muscle products once removed from their protected master packaging environment. The use of antioxidant application in the case-ready consumer market could be of significant impact as the industry reverts back to a traditional overwrap packaging system. Master packaging systems could include an antioxidant into the case ready package to extend the needed case life characteristics. Antioxidants including BHA and BHT are already utilized as food additives in many processed meat products throughout the processed meat industry. An antioxidant application system that could improve the shelf life and color stability of beef retail cuts could increase the profitability of the meat industry greatly. Steaks applied with the SYN treatment did maintain visual and objective color over an extended display day period with an increased postmortem aging period. The rate of lipid oxidation was also decreased with SYN treatment which suggests that retail case life could be extended. Given the results of this study, BHA and BHT applied to the surface of whole muscle beef cuts appears to be particularly effective for extending the shelf life when compared to current practices. The use of antioxidant application in the case-ready consumer market could prove useful as a greater amount of product is conveyed by traditional overwrap packaging systems. Master packaging systems could include an antioxidant application that would potentially increase desirable case life characteristics. Future research could examine effects of extended periods of display as the current study evaluated retail display for only five days within. Since there is such wide range and forms of both synthetic and natural antioxidants available, other potential antioxidant formulation could be considered. The overall consumer acceptance is not yet known and needs to be further addressed before industry applications are implemented.

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Huanglongbing and the California Citrus Industry: A Cost Comparison of Do Nothing vs. Do Something Management Practices

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ABSTRACT

The disease Huanglongbing (HLB) was first discovered in the United States in Florida in 2005. Since its discovery, HLB has not only decreased citrus production, but has drastically increased production costs. With California contributing over 80% of the nation's fresh oranges, it is important to attempt to keep HLB from becoming endemic in this state. This study examines two alternative management practices and estimates the potential total loss in production value over a 20-year period due to HLB in the California citrus industry. The total loss is estimated to be \$2.7 billion under a do-nothing approach and \$2.2 billion under an aggressive mitigation approach. This suggests that limiting the spread of HLB is the preferred management approach. It not only results in total damage savings of \$2,803 per acre over the do-nothing approach, but also protects the California citrus industry from HLB and promotes economic growth.

KEY WORDS: Huanglongbing, citrus greening, fresh oranges, damage costs, production costs, management practices

INTRODUCTION

The disease Huanglongbing (HLB), also known as citrus greening, was first discovered in the United States in Florida in 2005. Two positive tests were confirmed by the United States Department of Agriculture (USDA) in Miami-Dade County in southern Florida on September 2, 2005 (Animal and Plant Health Inspection Service 2005). These detections initiated changes in traditional management practices and domestic trade policies and regulations, and prompted the allocation of millions of dollars for related research funding.

HLB is a bacterium that affects all citrus cultivars. From the genus *Candidatus Liberibacter*, this phloem-limiting, gram-negative bacterium inhibits the flow of nutrients throughout the tree, decreases fruit production (Bové 2006; Citrus Research Board 2011), and can kill the tree (Citrus Research Board 2011). Oftentimes, isolated limbs of the tree exhibit symptoms and limb dieback diminishes production. Root loss before detecting symptoms can also lead to production losses. In general, as the health of the tree declines, early fruit abscission increases while the fruit also becomes bitter and misshapen, and

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remains green and small (USDA 2011a). Ultimately, the fruit is undesirable and unmarketable.

Within five to eight years of infection, the tree will no longer be economically productive (National Research Council 2010). When attempting to limit the spread of HLB, infected trees are usually removed before they ever reach the point of being considered unproductive. There is no cure for this disease, so prevention is important. Severe symptoms may appear in young trees as early as 6 months post infection and typically from 1-5 years for mature trees (National Research Council 2010, p. 4).¹ This potential for delayed symptom expression adds to the threat of this disease.

HLB generally transfers from tree to tree through three different means: the Asian citrus psyllid (ACP), the African citrus psyllid, and contaminated budwood propagation. Without any of these transmission methods present, the spread of HLB is limited. While the African citrus psyllid is considered a vector pest of HLB, it is not currently found in the United States and therefore is not a current concern in California. The Asian citrus psyllid, on the other hand, is found in the United States and has been a major concern in every citrus growing state; especially in Florida and California, the leading citrus producing states. In Florida where HLB is endemic, regulations have been placed to minimize the transfer of HLB through propagation. For instance, nursery stock may only come from trees that test negative for the bacteria and are grown in screened nursery buildings.

The Asian citrus psyllid (*Diaphorina citri*) is the main vector of HLB transmission. Since its introduction to the United States in 1998, ACP has been found in ten states, including Alabama, Arizona, California, Florida, Georgia, Hawaii, Louisiana, Mississippi, South Carolina, and Texas. Acting in the capacity of a vector of HLB, this species of psyllid has the potential to spread the disease anywhere the psyllid goes.

ACP was first detected in California in 2008 (Blake 2008). California supplies the United States with over 80% of its fresh oranges and is the country's largest exporter of fresh citrus. While Florida has a larger amount of acreage dedicated to citrus than California, in 2009 California contributed 45% of the industry's nearly \$2.9 billion value of production (USDA 2011b).

General and Specific Objectives. The main objective of this study is to simulate the potential economic impact of HLB on the California citrus industry. The specific objectives of the study are: (a) to determine and estimate the costs involved with limiting the spread of HLB in California; (b) to estimate and compare the net present value of the total damage costs due to HLB over a period of 20 years for two management practices; and (c) to evaluate and compare the total production loss in oranges in California due to HLB for these the two management practices.

¹ Bové (2006, p. 14) discusses the appearance of severe symptoms according to various forms of HLB (Asian, African, and American), temperature conditions (hot, warm, and cool), and altitudes (low and high). In Bové (2006, p. 14), severe symptoms were usually obtained after 7.5 months. Bové (2006, p. 29) also explains that the latency period during which recently infected trees do not show symptoms may vary from tree to tree, and is generally assumed to be 6 to 12 months long, if not longer.

MATERIALS AND METHODS

The study analyzes two management approaches to estimate the potential economic impact of reduced yields and increased costs associated with HLB management in California. The first management approach consists of no attempts to limit the spread of the disease when HLB is introduced into California. HLB is projected to spread rapidly throughout the state under this management approach. This management approach is referred to as the pessimistic approach. On the contrary, the second management approach attempts to minimize the HLB spread throughout the state when HLB is introduced into California. Both the pessimistic and optimistic approaches estimate the decline in yields over time and the costs associated with attempting to minimize the spread of HLB.

ACP is currently found in California; and although it may not be too late to eradicate ACP from the entire state, it would be difficult to accomplish since ACP is found in Mexico and Arizona, which makes reintroduction likely to occur. In order to analyze how HLB may impact the orange production in California, this study assumes that ACP spreads through the state, as was seen in Florida, and becomes a naturalized species. ACP naturalization means that once HLB is introduced, it has the potential to spread through the entire state.

If farmers were to make no changes in cultural practices after an introduction of HLB, the disease would ultimately reduce yields as it spreads through the state. It would be difficult to keep production levels high enough to be economically productive. Attempting to replant trees would also be ineffective, as young trees are very susceptible to infection and may never produce any fruit. Therefore, the pessimistic approach assumes no replanting attempts.

Given the California citrus industry's contribution to the state economy and the fact that many growers depend on it to make a living, a do-nothing approach is likely to be rejected by the majority of growers. According to Morris and Muraro (2008) and Roistacher (1996), if effective control practices are followed diligently, it is possible to keep the rate of greening infection below 1%. With ACP populations established throughout the state, the optimistic approach assumes immediate attempts to limit the spread of the disease will take place upon the discovery of HLB. This includes beginning to conduct HLB field surveys and increasing pesticide applications in an attempt to minimize ACP population levels throughout the state. As HLB spreads throughout California, there would be costs associated with diseased tree removal and replacement, and yields would also decrease. While the extent to which control practices will be implemented diligently depends on each grower, the optimistic approach assumes that all growers in the state will take an active role. Expenses incurred by the state of California in monitoring that growers take such active role can be incorporated into the analysis (as in Miranda et al. 2013). However, since there is little (if any) information in the US on state-wide monitoring expenses, our optimistic approach does not take into account such expenses.

The loss in production value under a worst case scenario (a do-nothing approach) can be compared with optimistic scenario (a do-something approach) to assist producers in their decision making process and assess policy implications. For instance, such comparison provides an estimate of the additional damage that could be avoided by adopting modified cultural practices.

Production Loss. The total production loss (\$/acre) in year t (TL_t) is estimated as

$$TL_t = HP_t - HLBP_t, \quad (1)$$

where HP_t and $HLBP_t$ are the production (\$) in year t without and with the presence of HLB respectively.

The healthy production (\$) in year t (HP_t) without the presence of HLB is estimated as

$$HP_t = (HY_t \times k_t \times P_{pt}) + (HY_t \times (1 - k_t) \times P_{ft}), \quad (2)$$

where HY_t is the expected yield (75-pound cartons per acre) from healthy trees at year t , k_t is the proportion of oranges that will be processed in year t , P_{pt} is the price of processed oranges (\$ per 75-pound carton) in year t , $(1 - k_t)$ is the proportion that will be sold as fresh oranges in year t , and P_{ft} is the price of fresh oranges (\$ per 75-pound carton) in year t .

On the other hand, the production (\$) in year t under the presence of HLB ($HLBP_t$) is estimated as

$$HLBP_t = P_{ft} \times HLBY_{ft} + P_{pt} \times HLBY_{pt}, \quad (3)$$

where $HLBY_{ft}$ and $HLBY_{pt}$ are the expected yields (75-pound cartons per acre) under the presence of HLB that are sold as fresh oranges and processed in year t respectively. These variables are estimated as

$$HLBY_{ft} = HLBTY_t - HLBY_{pt} \quad (4)$$

and

$$HLBY_{pt} = HLBTY_t \times k_t. \quad (5)$$

Total yield (75-pound cartons per acre) under the presence of HLB ($HLBTY_t$) is estimated differently under the pessimistic and optimistic approach.

The present value of the total damage cost (\$/acre) (PV_{Damage}) from HLB over a 20-year period is simulated 10,000 times using equation (6).² That is,

$$PV_{Damage} = \sum_{t=1}^T (1 + i)^{-t} \times D_t, \quad (6)$$

where D_t is the total damage costs (\$/acre) from HLB in year t and is estimated differently under the pessimistic and optimistic approach, as explained in the next two subsections.

² According to the National Research Council (National Research Council 2010, p. 121), the lifespan of a citrus tree can reach 100 years. A 20-year scenario is consistent with the remaining lifespan of several orange groves in California. Several studies have used a 20-year time period (Miranda et al. 2012; Bassanezi and Bassanezi 2008). Miranda et al. (2012) explain that they chose a 20-year period because 20 years is the life expectation of citrus orchard in the Sao Paulo, Brazil.

Pessimistic Approach Model. Under pessimistic approach, the total damage costs (\$/acre) from HLB (D_t) in year t equals the total production loss (\$/acre) from HLB in year t (TL_t). That is,

$$D_t = TL_t, \quad (7)$$

where TL_t is estimated by equation (1).

In addition, total yield (75-pound cartons per acre) under the presence of HLB and is estimated as

$$HLBTY_t = HY_t \times RY_t, \quad (8)$$

where RY_t is the relative yield at year t . A negative exponential model is used to estimate RY_t (Miranda et al 2012; Bassanezi et al. 2011; Bassanezi and Bassanezi 2008). That is,

$$RY_t = e^{(-1.8TD_t)}, \quad (9)$$

where TD_t is total disease severity at year t . Equation (9) is used to compare HLB yields with yields from healthy trees (Miranda et al. 2012, Bassanezi et al. 2011, Bassanezi and Bassanezi 2008). Producers usually stop harvesting infected trees before relative yield reaches very low levels, as returns to growers will no longer cover the cost of production and/or harvesting.

Total disease severity (TD) can be estimated as

$$TD_t = \sum_{j=0}^{j=t} (y_j - y_{j-1}) s_{t-j}, \quad (10)$$

where y is the incidence of symptomatic trees and s is the portion of the canopy exhibiting HLB symptoms (Bassanezi and Bassanezi 2008). Equation (10) combines the proportion of HLB severity in individual trees (s_t) and the incidence of symptomatic trees (y_t) and can be used to estimate the overall severity of HLB in a grove for any number of years (t) after an HLB introduction.

The incidence of symptomatic trees (y) in year t is estimated using the Gompertz function (Bassanezi and Bassanezi 2008; Miranda et al. 2012),

$$y_t = e^{-(-\ln(y_o))e^{-Rt}}, \quad (11)$$

where y_o is the portion of symptomatic trees when HLB symptoms first present themselves and R is the rate of disease incidence progress through a grove each year. R is estimated to range from 1.3 for trees between 0 and 2 years old down to 0.244 for trees older than 10 years (Bassanezi and Bassanezi 2008).

Determining the rate of spread of a pest and or disease is a challenge. Different environmental factors can attribute to how rapidly and how successful an invasive species or pathogen can be, in addition to the number of original pest introduction sites. Management practices play an important role in the dispersion of HLB over time. Aggressive management practices may allow contaminated groves to stay economically viable.

The proportion of HLB severity in an individual tree (s) is approximated by

$$s_t = \frac{1}{1 + \left(\frac{1}{s_0} - 1\right)e^{-rt}}, \quad (12)$$

where s_0 is the initial proportion of symptom expression, and r is the rate at which HLB moves through the tree in year t (Bassanezi and Bassanezi 2008; Miranda et al. 2012). The initial disease severity may change how fast HLB progress through a tree. The age of the tree plays a critical role in the rate of disease spread. HLB is estimated to progress through young trees ($r = 3.68$) at such fast rates that the tree may never become productive. When no control practices are in place, the high rate of spread in young trees makes replanting ineffective. Young trees are highly likely to become infected shortly after planting. The initial proportion of symptom expression (s_0) is estimated to range from 0.2 to 0.025 depending on the age of the tree (Bassanezi and Bassanezi 2008), while the rate at which HLB moves through the tree (r) is estimated to range from 3.68 down to 0.69 depending on the age of the tree (Bassanezi and Bassanezi 2008).

A two-year-old tree is estimated to get infected in less than two years, whereas a 10-year-old tree can take up to 10 years. Once a tree is infected its production will decline. An infected tree can continue producing, but the fruit quality is likely to be degraded.

There are currently around 180,000 bearing acres of oranges in California (USDA 2011b). Of these 180,000 acres, approximately 86% are over 10 years old and 9% fall into the age category of 6 to 10 years old (California Agricultural Statistics Service 1999, 2002; USDA 2006a, 2008a, 2010a, 2011b; Computed by Author). Instead of assuming that the entire grove is in the same age bracket (Bassanezi and Bassanezi 2008), this study accounts for the variability in the age of trees by using a stochastic simulation model. A stochastic simulation model takes into account different parameters from each age group that determine the rate of spread of HLB and estimates the total expected damage of HLB over time.

The proportion of symptom expression in individual trees (s_0), annual rates of HLB progress in individual trees (r), and the annual rate of HLB incidence progress through a block of oranges (R) are estimated using PERT distributions. With most of trees in California being 10 years or older, the minimum and most likely values of the PERT distribution are appropriately estimated by the parameter values that correspond to the trees greater than 10 years old (Bassanezi and Bassanezi 2008).

Optimistic Approach Model. Under the optimistic approach, the total damage costs (\$/acre) from HLB (D_t) is the sum of the total loss in production value per acre (TL_t) plus the additional costs associated with limiting HLB spread per acre (AC_t). That is,

$$D_t = TL_t + AC_t, \quad (13)$$

where TL_t is estimated by equation (1), and AC_t is the total additional costs incurred per acre in year t as a result of HLB. The variable AC_t is estimated as

$$AC_t = \Delta FC + RT_t + PT_t, \quad (14)$$

where ΔFC is the immediate per-acre increase in fixed production costs;³ RT_t is the cost of removing the tree in year t , equation (15), which depends on the rate of spread of HLB; and PT_t is the total per acre cost of replanting trees in year t that were removed the previous year, equation (16).⁴

The direct cost of removing the tree (RT_t) is estimated as

$$RT_t = CR \times OTL_t, \quad (15)$$

while and the direct cost of replacing it (PT_t) is estimated as

$$PT_t = CP \times OTL_{t-1}, \quad (16)$$

where CR is the tree removal cost, CP is the cost of a replacement tree, and OTL_t is the number of orange trees that were removed/loss in year t and is estimated as $(R_t \times HY_t)/(HY_t/121)$. It is assumed under the optimistic approach that all trees removed in year t are replaced with new trees the following year. The variable OTL_t depends on the rate of HLB spread (R_t) in year t , which is estimated using a PERT distribution (Cook et al. 2007, Cook and Matheson 2008). The minimum, most likely, and maximum values of the PERT distribution for R_t differ in the optimistic approach and pessimistic approach. The optimistic approach assumes that orange growers attempt to keep the HLB spread rate as small as possible.

The minimum, most likely, and maximum values of the PERT distribution for R_t under the optimistic approach are assumed to be 0.010, 0.023, and 0.032 respectively. According to Morris and Muraro (2008) and Roistacher (1996), the rate of HLB spread can be kept as low as 0.010. According to Morris et al. (2008) and Morris and Muraro (2008), the rate of spread of HLB in Florida averages 0.023 when attempting to limit its spread. The National Research Council (2010) reports a rate of spread as high as 0.040. Given that the 4% reported by the National Research Council (2010) correspond to mixed management practices and no uniform attempt to control the HLB spread, the maximum value for R_t is assumed to be 0.032.

Finally, total yield (75-pound cartons per acre) under the presence of HLB and is estimated as

$$HLBTY_t = HY_t - DY_t - DY_{t-1} - DY_{t-2} - DY_{t-3} - DY_{t-4} - DY_{t-5}, \quad (17)$$

where DY_t is the yield reduction (75-pound cartons/acre) from removing diseased trees in year t . It usually takes from four to five years for the replacement tree to produce oranges.

³ According to the California Agricultural Statistics Service (1999, 2002) and USDA (2006a, 2008a, 2010a, 2011b), approximately 86% of the orange trees in California are over 10 years old. Given that most of the orange trees in California are already grown up, this study assumes of a fixed increase in fixed production costs (a fixed increase in scouting costs and pesticide applications).

⁴ The costs that are taken into account consist of scouting for the disease, removing the diseased trees in year t (RT_t), replanting removed trees with replacement trees in year t (PT_t), and managing ACP, which mainly consists of applying additional pesticides. Clearly, not all the costs associated with the presence of HLB are included in this assessment. Some additional costs derived from HLB establishment, including managerial and management implementation costs, are not included in the analysis, as there is little (if any) information available about these costs.

In addition, DY_t takes into account the opportunity cost of removing diseased but still productive trees. It is estimated by multiplying the healthy yield in year t (HY_t) by the proportion of trees that are removed in one acre.⁵ That is,

$$DY_t = \frac{OTL_t}{121} \times HY_t . \quad (18)$$

Estimated ACP Control, Scouting, Tree Removal, and Tree Replacement Costs. The cost of controlling ACP populations depends on the insecticide that is used and the number applications. However, there seems to be disagreement in the number of insecticides applications that are needed to manage greening. For instance, some studies explain that many large growers in Florida have used systemic insecticides such as Temik (whose chemical name is aldicarb and its use on citrus was discontinued effective December 31, 2011 (Bayer Crop Science, 2010)) for mature trees and Admire (whose chemical name is imidacloprid) for young trees; and spraying at least five times a year (three sprays are in addition to the grove's regular spray program) to manage greening (Morris et al. 2008; Rogers et al. 2008). On the contrary, other studies explain that monthly or fortnightly insecticide applications (12 to 24/year) have not been enough to keep the disease under control or at a constant rate, especially in young groves (Gatineau et al. Fruits 2006; Bassanezi et al. 2013; Hall et al. 2013). In addition, it is now suggested to use imidachloprid and thiamethoxam instead of aldicar. The United States Environmental Protection Agency (EPA) and Bayer CropScience, the manufacturer of aldicarb, reached an agreement to end the use of aldicard in the United States. "To address the most significant risks, Bayer has agreed to first end aldicarb use on citrus and potatoes, and will adopt risk mitigation measures for other uses to protect groundwater resources. The company will voluntarily phase out production of aldicarb by December 31, 2014. All remaining aldicarb uses will end no later than August 2018" (US EPA 2010).

A recent study conducted in Sao Paulo state, Brazil (Belasque et al. 2010) reports costs for three HLB-management programs (program I: four inspections, five ground sprays, and one systemic insecticide; program II: six inspections, 10 ground sprays, two systemic insecticide, and one airplane spay; and program III: 12 inspections, 15 ground sprays, three systemic insecticide, and three airplane sprays). The total cost of programs I, II, and III in Sao Paulo State, Brazil were estimated to be \$96.38/acre, \$242.60/acre, and \$420.81/acre respectively (Belasque et al. 2010). Other studies estimate controlling for ACP in Florida can range from about \$400/acre to \$450/acre (Muraro 2010; Roka et al. 2010). Provided that the number of applications can vary significantly, and the fact that the optimum number of spray applications in Florida is unknown (Morris et al. 2008; Morris and Muraro 2008), this study estimates the average cost for pesticide applications and scouting in California to be around \$426.47/acre per year.

Since HLB is not currently found in California, there are no values that can be attributed to scouting costs. To estimate scouting costs, this study assumes that the costs are going to be similar as the ones in Florida. In 2011, the minimum wages in Florida and California were \$7.25 and \$8.00 per hour respectively (US DOL 2012). Florida's

⁵ Other factors such as early fruit drop off and reduced initial fruit set can also contribute to reduced yields under the presence of HLB. However, since the optimistic approach assumes that all trees identified with HLB will be immediately removed, these latter forms of yield loss are not considered in the analysis.

scouting costs range from \$14.00 to \$35.00 per acre (Morris and Muraro 2008). Following the principles of purchasing power parity (PPP), California's scouting costs are estimated to range from \$15.45 to \$38.62 per acre. If scouting is conducted four times per year as recommended (Morris et al. 2008; Belaque et al. 2010), scouting costs in California are estimated to average \$108.88 per acre per year.

The costs associated with tree removal vary depending on the number of trees that are being removed. Tree removal includes uprooting the tree, disposing of the tree, ground preparation for replanting the tree, and the direct cost of the replacement tree. The number of trees planted per acre can vary; however, this study assumes an average of 121 trees per acre. Removed trees will be replaced with new plantings the year after their removal. Removal and ground preparation costs are estimated at an average of \$13.34 per removed tree (Irey et al. 2008; Morris and Muraro 2008; O'Connell et al. 2009). The current cost of replacement trees in California is estimated at \$10.50 per tree (O'Connell et al. 2009). Since the introduction of HLB in Florida, the cost of replacement trees in Florida has doubled (National Research Council 2010). This study estimates the cost of replacing trees in California at \$21.00 per replacement tree. It has been shown that under consistent and stringent management practices for HLB, an average increase in tree removal of 2.3% is possible (Morris et al. 2008).

Estimated Prices, Yields, and Utilization. Under both the optimistic and pessimistic approach, the prices for both fresh and processed oranges for each year are estimated using price ranges from 2001 to 2008 and are incorporated in the simulation. The processed-orange price ranges from \$0.23 to \$1.52 per 75-pound carton while the fresh-orange price ranges from \$9.26 to \$18.01 per 75-pound carton (USDA 2003, 2004, 2005, 2006b, 2007, 2008b, 2009, 2010b). Based on the 1992-2011 annual fresh orange prices in California, the fresh orange price is assumed to be normally distributed with a mean of \$12.73 and a standard deviation of \$1.16 from year 1 to year 20 in the simulation analysis. Similarly, based on the 1992-2011 annual processed orange prices in California, the processed orange price was assumed to be normally distributed with a mean of \$0.76 and a standard deviation of \$0.25 from year 1 to year 20.

The expected yield is estimated using uniform distribution with a minimum value of 242 75-pound cartons per acre and a maximum value of 354 75-pound cartons per acre. The minimum and maximum yield values for the uniform distribution were determined based on the observed yields during crop years 2000-2011 (USDA 2002-2011).

To account for the variability in what percentage of the harvested crop goes to processing and what remains as fresh fruit under both approaches, a range of values for the percent that are processed is considered. In the last 10 years (period 2000-2011), the average minimum value for the percentage of oranges that are processed is 13.0% and the average maximum value is 29.1% (USDA 1994, 1995, 1996, 1997, 1998, 1999, 2000, 2001, 2002, 2003, 2004, 2005, 2006b, 2007, 2008b, 2009, 2010b, 2011b). Based on the observed values from 1994-2011, the proportion of oranges that will be processed is assumed to be uniformly distributed.

RESULTS

If HLB is allowed to spread throughout California without any attempts to limit it (the pessimistic scenario) for a period of 20 years, today's total loss in production value

is on average estimated to be \$2.7 billion. However, if California orange growers take aggressive actions attempting to limit its spread (the optimistic scenario), today's total damages over the 20-year period considered are on average estimated to be \$2.2 billion. When comparing the loss in production value between the pessimistic and optimistic approaches over a 20-year period, damages under the pessimistic approach are much higher (Table 1). Limiting the spread of HLB is the preferred approach and is consistent with several studies about citrus disease controls (Miranda et al. 2012; Salifu et al. 2012; Fishman et al. 1983). Under the pessimistic approach, it takes longer to see the reduction in yields while under the optimistic approach yield loss and increased production costs are observed sooner. The difference in the yield loss is related to the fact that under the pessimistic approach, infected trees are left to continue to produce, while under the optimistic approach, infected trees are removed immediately upon disease detection.

Compared to the simulated average value of healthy production, the estimated production loss accounts to 33% and 27% under the pessimistic approach and optimistic approach respectively. When compared directly to today's value of the past 20 years of production, this is equivalent to an estimated 19% and 15% reduction in the present value over the next 20 years if HLB were to be detected in 2012 for the pessimistic and optimistic approaches respectively. If total orange bearing acreage in California is held constant at 180,000 acres over the past 20 years, as in the 20-year-simulated projection, an estimated 26% and 21% decrease in production value is estimated under the pessimistic and optimistic approach respectively.

CONCLUSION AND DISCUSSION

With California contributing over 80% of the nation's fresh oranges, it is important to keep HLB from becoming endemic in the state. Quantifying the potential economic impact of HLB under different management approaches provides insight in developing the most appropriate mitigation practices and reinforces the importance of the actions and efforts of Plant Protection Agencies in preventing the introduction and establishment of HLB in California. The citrus industry in California is worth protecting from the spread of diseases. In 2009, California contributed 45% of the United States citrus industry's nearly \$2.9 billion production value (USDA 2011b). As California's 15th ranked commodity in terms of production value, orange production is worth an estimated \$722 million in 2010 (USDA 2011c) and employs around 26,000 people in the state (Chavez 2010).

This study approximates and compares the loss in orange production value in California due to HLB under a pessimistic and an optimistic scenario. The pessimistic approach estimated the costs associated with a do-nothing management practice while the optimistic approach estimated the costs associated with attempting to limit the spread of HLB (a do-something management practice). Monte Carlo simulations were employed to estimate the total damage of HLB in California under both approaches.

Fig. 1 and Fig. 2 report the average total loss in production value as well as 95% confidence intervals under each management approach over a 20-year period. Damages under the pessimistic approach tend to be higher but they are also more dispersed. On the contrary, damages under the optimistic approach tend to be smaller and have narrower confidence intervals. Average total damage under the pessimistic approach is estimated to be \$14,938 per acre (Fig. 1), while under the optimistic approach is estimated to be \$12,135 per acre (Fig. 2). This is a difference of \$2,803 per acre over the 20 years that are

projected. Under the pessimistic approach it takes longer to see the reduction in yields while under the optimistic approach yield loss and increased production costs are observed sooner (Table 1).

The optimistic approach considers an increase in pesticide applications, which leads to an increase in production costs, which could potentially lead to a decrease in orange acreage. Although production costs increase, the total damage caused by HLB is significantly less under the optimistic approach than in the pessimistic approach. About half a billion dollars in production costs savings is estimated over a period of 20 years if HLB is detected in California and the optimistic approach is chosen over the pessimistic approach. This suggests the optimistic approach is the preferred approach, which is also consistent with several previous studies about citrus disease controls (Miranda et al. 2012; Salifu et al. 2012; Fishman et al. 1983).

Under the pessimistic approach, production decreases by over 50% after 11 years following an HLB introduction. The production loss would be felt throughout the food distribution channel (chemical companies, growers, farmers, packing houses, distributing companies, marketers, etc.). Under the optimistic approach, the growers are likely to absorb the additional production costs but it is always possible that they could also be partially subsidized by the government as in Brazil (Miranda et al. 2012). If the government or the state of California does not help growers, the optimistic approach could also result in a partial reduction of the number of orange bearing acreage. Among the two management approaches considered (doing nothing vs. doing something), limiting the spread of HLB is the preferred management practice. It results in damage savings, protects the California citrus industry from HLB, and promotes economic growth. Further research could explore additional management practices or combinations of them.

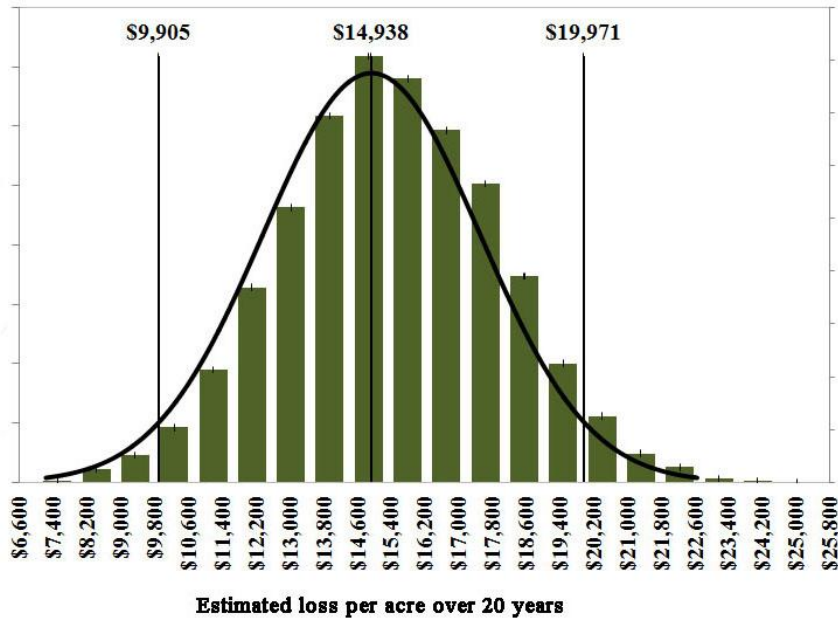


Figure 1. Distribution of the per acre loss in production value under the pessimistic approach.

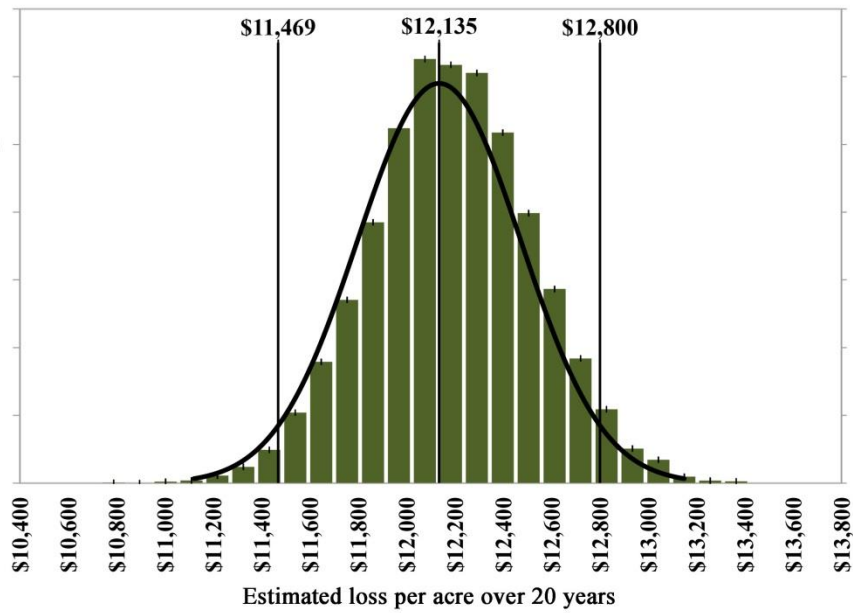


Figure 2. Distribution of the per acre total damage under the optimistic approach.

Table 1. Comparison of the average total damages and differences in average production losses per acre under the optimistic and pessimistic approaches.

Year	Optimistic Approach			Pessimistic Approach	Difference	
	Average Total Damage	Average Increased Production Costs	Average Production Loss	Average Total Damage	Production Loss	Total Damage
1	\$504.94	\$445.57	\$59.37	\$0.32	\$59.05	\$504.62
2	\$596.53	\$480.78	\$115.75	\$2.18	\$113.57	\$594.35
3	\$635.02	\$466.77	\$168.24	\$9.27	\$158.98	\$625.75
4	\$670.27	\$452.99	\$217.28	\$28.76	\$188.52	\$641.51
5	\$703.70	\$439.75	\$263.95	\$70.95	\$193.00	\$632.75
6	\$734.44	\$427.11	\$307.33	\$146.65	\$160.68	\$587.79
7	\$713.65	\$414.61	\$299.04	\$262.88	\$36.16	\$450.77
8	\$692.04	\$402.59	\$289.45	\$415.54	\$126.09	\$276.50
9	\$671.50	\$390.78	\$280.72	\$592.61	\$311.89	\$78.89
10	\$651.28	\$379.24	\$272.04	\$772.69	\$500.66	\$121.41
11	\$632.52	\$368.14	\$264.38	\$942.97	\$678.59	\$310.45
12	\$614.73	\$357.62	\$257.11	\$1,086.17	\$829.06	\$471.44
13	\$596.98	\$347.23	\$249.75	\$1,195.64	\$945.89	\$598.66
14	\$579.34	\$337.06	\$242.28	\$1,280.16	\$1,037.88	\$700.82
15	\$562.44	\$327.24	\$235.19	\$1,329.09	\$1,093.89	\$766.65
16	\$546.02	\$317.64	\$228.38	\$1,361.71	\$1,133.33	\$815.69
17	\$530.05	\$308.40	\$221.65	\$1,371.76	\$1,150.12	\$841.71
18	\$515.09	\$299.53	\$215.56	\$1,368.53	\$1,152.97	\$853.44
19	\$499.39	\$290.73	\$208.66	\$1,358.45	\$1,149.79	\$859.05
20	\$484.72	\$282.27	\$202.45	\$1,341.59	\$1,139.13	\$856.86
Total	\$12,134.64	\$7,536.07	\$4,598.57	\$14,937.91	\$12,159.24	\$11,589.13

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