

EFFECTS OF TANNIC ACID (BYPRO®) ON GROWTH PERFORMANCE,
CARCASS CHARACTERISTICS, APPARENT TOTAL TRACT DIGESTIBILITY,
FECAL NITROGEN VOLITALIZATION, AND MEAT LIPID OXIDATION OF
STEERS FED STEAM-FLAKED CORN BASED FINISHING DIETS

By

MELISSA CATHERINE TABKE, B.S.

A THESIS

In

ANIMAL SCIENCE

Submitted to the Graduate Faculty
of Texas Tech University in
Partial Fulfillment of
the Requirements for
the Degree of

MASTERS OF SCIENCE

Jhones O. Sarturi
Chair of Committee

Sara J. Trojan

J. Chance Brooks

Mark A Sheridan
Dean of the Graduate School

December, 2014

Copyright 2014, Melissa C. Tabke

ACKNOWLEDGEMENTS

First, I would like to thank Dr. Jhones Sarturi of the opportunities he has given me here at Texas Tech. He is an incredible teacher, has provided me with a learning environment unlike any other, and encourages excellence in all walks of life. I would like to thank my committee members Dr. Trojan and Dr. Brooks. I am very appreciative of the time and effort they have devoted to my academic career. A special thank you to Dr. Trojan for all the help and encouragement she has given me during my time here at Texas Tech.

Secondly I would like to thank all the students, interns, and staff who have helped me during this last crazy year. Specifically, Alex Thompson , Pedro Campinili, Katie Crossley, Jessica Baggerman, Fernando Varagas, Tyler Davis, Heather Rode, Megan McCullough, Olivia Ron, Tanner Schmidt, Kate Sharon, and Kishor Gautam. I cannot thank you all enough for helping me in the lab, in the meat cooler, and out at the feedlot. A very special thank you to Jennifer Martin who always made more time for me than she should have and helped guide me through the meat science portion of my project on a daily basis. Additionally, I would like to thank Kirk Robinson and Ric Rocha for all their attention to the care of my cattle and their help with all other feedlot matters.

Lastly thank you to my parents. To my dad Bruce Tabke for his inspiration, encouragement, and his ability to let me make my own mistakes, and to my mom Catherine Friesen for her love and support.

TABLE OF CONTENTS

ACKNOWLEDGMENTS..... ii

ABSTRACT..... v

LIST OF TABLES..... viii

I. INTRODUCTION..... 1

II. REVIEW OF LITERATURE..... 3

 Characterization of tannins..... 3

 Effects of tannins on ruminant digestion..... 6

 Effects of tannins on performance and carcass characteristics..... 8

 Effects of tannins on animal health..... 11

 Effects of tannins on meat quality..... 13

III. MATERIALS AND METHODS..... 17

 Cattle receiving and processing..... 17

 Experimental design and dietary treatments..... 18

 Management, feeding, weighing, and processing..... 19

 Harvesting and carcass measurements..... 21

 Apparent digestibility evaluation..... 22

 Fecal nitrogen volatilization..... 23

 Lab analyses of diets, refusals, feces, and pen surface samples..... 23

 Meat analyses..... 25

 Statistical analysis..... 27

IV. RESULTS AND DISCUSSION..... 29

 Cattle performance..... 29

Carcass Characteristics.....	33
Nutrient Intake and Digestibility.....	36
Fecal Nitrogen Volatilization.....	38
Meat Analysis.....	38
Tables and Figures.....	41
V. CONCLUSIONS.....	53
LITERATURE CITED.....	54

ABSTRACT

Effects of a tannic acid blend (ByPro®, Silva Team) added to steam-flaked corn (SFC) finishing diets on beef cattle growth performance, carcass characteristics, nutrient apparent digestibility, fecal nitrogen volatilization and meat lipid oxidation were evaluated. Steers (n = 144, initial BW = 349 ± 25 kg) were assigned to 1 of 3 treatments in a RCBD (12 pens/treatment; 4 steers/pen) and fed ad libitum, once daily for approximately 150 d. Treatments were designed as following: control (CON- no ByPro®), and ByPro® fed at 30 or 60 g of DM/steer daily (30-ByPro® and 60-ByPro®, respectively). Finishing diets based on SFC also contained wet corn gluten feed, alfalfa hay, cottonseed hulls, yellow grease, tannic acid ByPro® premix or cottonseed meal, urea, limestone, and a vitamin and mineral supplement. Initial BW was based on a non-shrunk limit-fed (1.8% of BW for 10 d) weight. Final BW, ADG, and G:F were based on HCW using current study average of dressing percent (62.14%) and corrected for a 4% shrink. Digestibility evaluation was performed on day 92-96 with acid insoluble ash used as internal marker to estimate fecal output. Air-dry surface fecal samples were collected 7-10 d after cattle shipment to harvesting facility, and N:P ratio used to estimate N volatilization (% N intake). Choice strip loins from two harvest groups were obtained. At 21 days of aging steaks were cut, overwrapped packaged, placed in a retail display case where instrument color data was taken. A second steak was also cut, frozen in liquid nitrogen and later evaluated for antioxidant activity in the meat. Data were analyzed using the GLIMMIX procedure of SAS with block (n = 12) as a random effect. Pre-planned contrasts were used to check for linear and quadratic effects of ByPro®

inclusion. Data from evaluations of meat quality traits were analyzed using a linear mixed model (PROC MIXED) of SAS. Harvest group was utilized as a random variable while carcass QG was incorporated as a covariate.

Intake quadratically increased ($P = 0.05$) from d 0 to 35 with ByPro® inclusion. Intake from d 0 to 105 ($P = 0.07$) and d 0 to end tended ($P = 0.06$) to increase linearly, with DMI by steers consuming 60-ByPro® being 3.7% greater than CON. ByPro® inclusion did not affect overall (d 0 to end) carcass-adjusted ADG ($P = 0.65$) or G:F ($P = 0.17$), averaging 1.62; 1.66; and 1.64 kg/d, and 0.163; 0.163; and 0.159 kg gain/kg DMI, for CON, 30-ByPro®, and 60-ByPro®, respectively. Carcass characteristics including, HCW (388 kg; $P = 0.52$), fat thickness (1.47 cm; $P = 0.32$), longissimus muscle area (94 cm²; $P = 0.57$), quality grade (88% upper-choice; $P = 0.44$), yield grade (3; $P = 0.29$), and liver score (15%; $P = 0.13$) were not affected by dietary inclusion of ByPro®. Inclusion of ByPro® tended to linearly influence intake of DM ($P = 0.07$) and OM ($P = 0.08$) during the digestion phase, while starch, CP, and NDF intake were not influenced ($P > 0.10$). Apparent digestibility of starch decreased linearly ($P = 0.03$) with ByPro® inclusion (1 percentage unit), while CP tended to decrease linearly ($P = 0.09$) and OM tended to decrease quadratically ($P = 0.09$) with inclusion of ByPro®. No differences were observed in fecal nitrogen volatilization as % of intake, g/steer/day, or kg/steer during the finishing phase. An increase ($P = 0.002$) in metmyoglobin in strip loin steaks was observed as inclusion of ByPro® increased. Only subtle differences were observed for L^* , a^* , b^* , hue angle, saturation, discoloration ratio or deoxymyoglobin were observed among treatments between display days, with the exception of L^* values ($P =$

018). There was an interaction ($P < 0.01$, SEM = 3.40) between all treatments and retail display day when analyzing oxymyoglobin. All values regardless of treatment declined as display progressed. However, on day 5 control and 30-ByPro® treatments had lower proportions of oxymyoglobin than steaks from 60-ByPro®. No differences were observed when pH and thiobarbituric acid reactive substances were analyzed. Tannic acid (ByPro®) seemed to have more influence on feed intake during the first half of the feeding period when added to beef cattle finishing diets. ByPro® decreased starch, CP, and OM digestion but increased DMI and OMI. ByPro® had no impact on fecal nitrogen volatilization. ByPro® inclusion seemed to have no ability to increase retail meat antioxidant activity but did not negatively affect meat quality.

LIST OF TABLES

1. Dietary ingredients and calculated nutritional composition of receiving and step-up diets fed to steers 41

2. Composition of the TTU mineral and vitamin supplement of experimental diets.....42

3. Dietary ingredient inclusion and analyzed nutritional composition of finishing diets containing levels of tannic acid (ByPro®) fed to steers.....43

4. Effects of ByPro® (tannic acid) titration (g/steer/day) in steam-flaked corn-based diets on beef cattle growth performance44

5. Effects of ByPro® (tannic acid) titration (g/ steer /day) in steam-flaked corn-based diets on beef cattle carcass characteristics.....45

6. Effects of ByPro® (tannic acid) titration (g/ steer /day) in steam-flaked corn-based finishing diets on beef cattle apparent total tract digestibility.....46

7. Effects of ByPro® (tannic acid) titration (g/ steer /day) on fecal nitrogen volatilization47

8. Instrument color values (L^* , a^* , b^* , hue angle and saturation), discoloration ratio, and relative pigment proportions (% oxymyoglobin, deoxymyoglobin, metmyoglobin) of overwrapped packaged *L. lumbrorum* steaks during retail display as influenced by inclusion level (0,30,60 g/steer/d) of ByPro® (tannic acid) into steam flaked corn based diets of finishing beef cattle.....48

9. The effects of retail display day on the instrument color values (L^* , a^* , b^* , hue angle and saturation), discoloration ratio, and relative pigment proportions (% deoxymyoglobin, metmyoglobin) of overwrapped packaged *L. lumbrorum* steaks from cattle fed steam flaked corn based diets with ByPro® (tannic acid) incorporated at 0, 30, or 60 g/steer/d49

10. Relative proportion of oxymyoglobin (%) during retail display in overwrapped packaged *L. lumbrorum* steaks from cattle fed steam flaked corn based diets with ByPro® (tannic acid) incorporated at 0, 30, or 60 g/steer/d50

11. Thiobarbituric acid reactive substances (TBARs; mg malondialdehyde/kg meat) in 21 d aged beef strip loins from cattle fed steam flaked corn based-diets with ByPro® (tannic acid) incorporated at 0, 30, or 60g/steer/d.....52

CHAPTER 1

INTRODUCTION

Tannins are found in many plants commonly included in ruminant diets such as alfalfa and sorghum (Frutos et al., 2012). They are a group of water soluble polyphenolic compounds that can complex with proteins, starches, and some vitamins and minerals in moderate pH, like in ruminal conditions, and dissociate at lower pH, like in abomasum and the initial portion of the duodenum (Porter, 1992). This ability to precipitate proteins and other nutrients, if correctly dosed, may possibly increase the digestibility and finally efficiency of utilization of nutrients as they are able to bypass ruminal digestion, decreasing ruminal losses, and become available at small intestinal level. This increase in digestibility and efficiency could lead to an increase in performance. Barajas et al. (2012a,b) observed that inclusion of a tannic acid blend (ByPro®, Silva Team) increased ADG by 0.2 kg and final BW by 12 kg of yearling bulls fed a ground-corn based diet. The ability of tannin to bind to protein may also decrease fecal nitrogen volatilization as some tannin-protein complexes will not completely dissociate during digestion. Tannins may also confer antioxidant activity due to its polyphenolic and flavonoid structure (Pennington and Fisher, 2009). Other antioxidants that are commonly found in plants, such as alpha-tocopherols, have been known to increase meat shelf life (Velasco and Williams, 2011). Cyaniding, a form of tannin has an oxygen radical absorbance capacity of around 4500 (Velasco and Williams, 2011) Thus it can be inferred that tannins may have the ability to induce similar effect. However, none of the potential positive effects of tannins cited above were evaluated for the tannins blend (ByPro®, Silva Team) fed in

steam-flaked corn-based finishing diets fed to beef steers. Therefore, the objective of this study was to evaluate the effects of a tannic acid blend (ByPro®, Silva Team) dietary titration on growth performance, carcass characteristics, apparent total tract digestibility of nutrients, fecal nitrogen volatilization, and meat lipid oxidation of finishing beef steers fed a steam-flaked corn-based finishing diets.

CHAPTER II

REVIEW OF LITERATURE

Characterization of tannins

Tannins are a group of water soluble polyphenolic compounds with a high molecular weight found in many plants that are included in ruminant diets (Frutos et al., 2012; van Soest, 1994). Their name was derived from French and German words that describe the bark of trees used to tan leather (Frutos et al., 2012; MacAdam et al., 2013). They are found not only in tree bark but also in many fruits, a wide variety of legumes (vetch, alfalfa, lentils, pericarp of peanuts), and grasses like corn and sorghum (van Soest, 1994). It is thought that tannins were developed by plants as a defense mechanism to protect against insects and herbivores (Bi et al., 1997). Generally, they are classified into two groups; hydrolysable and condensed or proanthocyanidins tannins (Reed, 1995). Tannic acids are commercially generally composed of various mixtures of hydrolysable tannins but can also be derived from condensed tannins (Hagerman, 2010).

Condensed tannins are made from a phenylalanine and polyketide biosynthesis derived heterocyclic ring system that is the basis for polymeric flavonoids (Hagerman, 2002). Some proanthocyanidins are responsible for red and blue hues of pigment, evident if leaf color change in the fall, and astringent tastes found in many flowers, wines, and fruits (Cannas, 2014; van Soest, 1994). They are found more readily in the new growth of plants that usually have the most nutritive value like new leaves, immature fruits, and flowers (Cannas, 2014; van Soest, 1994). Most condensed tannins are polymers of polyhydroxyflavan-3-ol monomers with flavan-3-ols (-)-epicatechin and (+)-catechin

being the more widely researched (Hagerman, 2002; Porter, 1992). These units can be linked via 4-6 or 4-8 carbon- carbon bonds (Porter, 1992). However, some condensed tannins can be further polymerized, like in sorghum, to yield linear 4, 8 polymers (Hagerman, 2002). These structures have a high molecular weight and an ability to chelate with some metal ions, thus potentially affecting the bioavailability of certain macrominerals like sulfur and iron (Santos-Buegla and Scalbert, 2000). This, combined with their ability to bind to proteins makes them good antioxidants (Santos-Buegla and Scalbert, 2000). Additionally, they have been speculated to help prevent cancers and cardiovascular diseases (Santos-Buegla and Scalbert, 2000).

Hydrolysable tannins are technically classified into two separate subcategories; either gallotannins or ellagitannins (Hartzfeld et al., 2002). Gallotannins, the simpler of the two types, hydrolyze into gallic acid, while ellagitannins hydrolyze into ellagic acid. However, ellagitannins are grouped with gallotannins in most literature. They are simply gallotannins that have groups of galloyls that have been oxidatively coupled with the ellagitannin (Hagerman, 2010). Tannic acid has been previously categorized as being only a hydrolysable tannin, being made up of a glucose and gallic acid (van Soest, 1994). At the center of hydrolysable tannins there is a glucose-based core (Hartzfeld et al., 2002). Pentagalloyl glucose (PGG) is the basis for most gallotannins and exists in many isomers, all of which have different chemical and biochemical properties which confer their ability to precipitate proteins (Hagerman, 2010).

Tannins tend to be stable in aqueous solutions but will dissolve in stronger acid or base solutions (Porter, 1992). While tannins have some interactions with starches,

cellulose, and some minerals, their main interactions for nutritional purposes are with proteins (Cannas, 2014). The large number of phenolic groups that tannins possess allow them to bind more readily with proteins as they have many points where the carbonyl groups of peptides can bind (Lorenz et al., 2013; McLeod, 1974). The higher the molecular weight and their structural flexibility are what allows certain tannins a higher binding affinity than others (McLeod, 1972; Mueller-Harvey and McAllan, 1992). Tannin-protein complexes are generally not very stable unless at pHs closer to neutral, as those found in the rumen (Lorenz et al., 2013). These tannin-protein complexes can degrade at lower pHs, closer to that of the abomasum and duodenum (Reed, 1995) and are also able to dissociate at more basic pHs (Porter, 1992).

Both hydrolysable and condensed tannins have the ability to form reversible and irreversible compounds with proteins that can decrease the amount of ammonia produced in the rumen as well as the amount excreted from the animal, making nitrogen usage more efficient (Lorenz et al., 2013). However, in large amounts (at or above 23.9% dietary DM (Woodward and Reed)), certain condensed tannins may decrease forage quality, especially if the forage is dried, allowing tannin-plant protein binding through oxidative cross-linkage, making that protein unavailable in the rumen environment (van Soest, 1994). During times of stress or near death, plants are able to increase the amount of anthocyanins in their tissues (van Soest, 1994). Tannins are also able to inhibit protein digestion when in excess as they can create an acid resistant tannin-protein binding that may decrease the ability of tannins to release the proteins in the small intestine and be degraded to amino acids for absorption (Reed, 1995; Lorenz et al., 2013). As tannins also

have the ability to inhibit enzyme activity, this may cause a decrease in protein digestion (van Soest, 1997) Hydrolysable tannins have been classically thought of as being very toxic to animals, especially if consumed in large amounts (Garg et al, 1992). More recent literature has shown that both tannins do reduce animal performance at larger amounts (23.9% dietary DM (Woodward and Reed, 1997)) but at smaller amounts (0.33% (Barajas et al, 2012) – 19.0% dietary DM (Woodward and Reed, 1997)), may be beneficial to protein digestion.

Effects of tannins on ruminant digestion

Tannins are a common component of many forage legumes and have been widely accepted as a component that may decrease digestibility. Research has been conducted with tannins as a way to bypass ruminal digestion and increase digestion and absorption of proteins in the small intestine. Hydrolysable tannins have always been thought of as being very toxic to rumen microorganisms. When hydrolysable tannins are degraded by rumen microbes, they produce pyrogallol which is a hepatotoxin and a nephrotoxin (Reed, 1995). They inhibit enzymes causing substrate deprivation, have negative effects on microorganism membranes, negative effects on growth, and also cause metal ion deprivation (Cannas, 2014; Makkar et al., 1988). They specifically have an effect on cellulolytic organisms (Mandels and Reese, 1963). However, it is suggested that ruminants are able to hydrolyze these types of tannins into gallic acid, which can be absorbed in the rumen and small intestine and further metabolized (Bhat et al., 1998; Selinger et al., 1996; Singh et al., 2001). The astringent taste that is produced when tannins bind to salivary mucoproteins and can increase saliva production which may help

buffer and increase liquid turnover rate in the rumen (van Soest, 1994). It has also been speculated that a partial adaptation to tannins can be achieved with their continuous inclusion in diets that can defer some of the negative effects of these compounds (Silanikove, 2000). Proline- rich proteins have an especially high binding affinity for tannin compounds (Mehansho et al, 1987). While still toxic when included at a high percent of dietary DM, this gives producers some leeway in deciding what type of feed and how much of that feed they are able to supply in a diet without tannin toxicity.

Condensed tannins, the more common type of tannin in forages, have avoided being labeled as toxic as they are not absorbed or degraded in the rumen; however, they have been known to cause lesions in the gut mucosa (Reed, 1995). As stated previously, all tannins are able to form complexes with not only proteins but some metal ions, microbial proteins, and also carbohydrates, which all impact the digestibility of nutrients.

Tannic acid has been shown to be able to be degraded into non-toxic acids by the microorganisms in the gastrointestinal tract (Bhat et al., 1998). However, many tannins are still generally resistant to most proteases, especially in more neutral pH conditions (van Soest, 1994). Some microorganisms are able to degrade tannic acid- protein systems and then further degrade the protein for absorption (Brooker et al., 1994).

Zhu et al. (1995) observed that when sheep were dosed with tannic acid, gallic acid and pyrogallol were found in the rumen fluid and plasma as well as urine. The concentration of pyrogallol continues to increase but the concentration of gallic acid decreasing at a gradual rate after dosing. They dosed tannic acid intra-uminally and found that it was directly correlated to liver necrosis. Authors also observed that when

sheep were dosed with tannic acid directly into the abomasum, the presence of liver and kidney necrosis developed. Authors speculated that this bereavement of the liver and kidneys was the result of un-metabolized tannic acid.

An in vitro study (Martinez et al., 2006) evaluating effects of tannic acid (used to represent hydrolysable tannins) and quebracho tannins (condensed tannin) on ruminal fermentation of wheat and corn grain showed that both types of tannins reduced gas production in both corn and wheat grains when included at 50 g/kg DM. There was subtle effect on total dry matter disappearance between tannin-treated and control with both corn and wheat over the entire study, however, in the first 12 hours after incubation 43% of untreated wheat was digested and only 22% of quebracho and 17% of tannic acid treated wheat was digested. At 24 hours of incubation, DMD of wheat was also significant, untreated wheat was being 85% digested and tannin treated wheat being 71 and 64% digested for quebracho and tannic acid treated wheat, respectively. Between tannin sources ammonia production did not differ. Total VFA production was less for tannin-treated samples than control samples with total VFAs and 12 hours being 75.48 mmolL⁻¹ and only 57.20 and 52.17 mmolL⁻¹ quebracho and tannic acid treated wheat, respectively. After 48 hours, untreated wheat produce 147.64 mmolL⁻¹ of total VFA production, while quebracho and tannic acid treated wheat only produced 126.59 and 126.45 mmolL⁻¹, respectively. As with wheat, corn VFA production also differed significantly, with control corn producing 120.39 mmolL⁻¹, quebracho treated corn producing 109.24 mmolL⁻¹ and tannic acid treated producing 104.77 mmolL⁻¹.

Effects of tannins on performance and carcass characteristics

Until more recently, it was widely believed that tannins had negative effects on animal intake and growth. Several more recent studies have shown that is not necessarily true.

Barajas et al. (2012a) observed that supplementing a mixed condensed and hydrolysable tannin blend in a sorghum based diet at approximated 33.6 g/d (approximately 0.3% of dietary DM) fed to bulls increased ADG and DMI by 0.214 and 0.693 kg/d, respectively. An increase in intake as a percent of BW (0.1% of BW) and a decrease in plasma urea nitrogen levels (2.75 mg/dL) were also observed. It should be noted that the amount of ground sorghum in the diet was increased to 61.83% in the finishing ration. Sorghum has a large amount of tannin, up to 136 mg/100g in high tannin varieties (Radhakrishman and Sivaprasad, 1980), therefore the amount of tannin fed to animals may have been up to 10.2 g/hd/d greater than that originally provided from blended source. Another study conducted by Barajas et al. (2012b) showed that ADG was positively effected by 0.187kg/d in bulls fed a dry-ground corn-based diet containing corn dry distillers grains and cane molasses when a blend of soluble and condensed tannins were included at 36g/hd/d or about 0.33% of dietary DM. Bulls fed the tannin blend also weighed an average of 14.73 kg more at the end of the 84 day feeding trial than bulls fed the control diet. However, net energy for maintenance and net energy needed for gain were inversely effected by tannin inclusion , NEm being 0.15 and NEg being 0.131 Mcal/kg great for tannin treated cattle. No other performance aspects were effected by tannin inclusion and carcass characteristics were not measured. Arechiga et al. (2011) showed that length of feeding blended soluble and condensed tannin-extracts

included at a 30 g/hd/d to finishing bulls on a ground corn based diet resulted in a linear decrease in back fat thickness from 10.73 to 9.26 mm when fed for 0 to 100% of the 102 day finishing period. They also showed a tendency for a linear decrease from 2.05% to 1.60% in KPH fat percentage from control cattle to cattle fed tannin blend all 102 days of the trial. However, length of feeding of tannin did not affect carcass characteristics, and growth performance measurements were not measured.

Another study looking at the effects of growth performance on feeding length of a blend of condensed and hydrolysable tannins at 0.32% of dietary DM in finishing feedlot bulls for 0, 68 or 100% of the finishing phase (Barajas et al., 2011a) showed that final body weight (14.95 kg), total weight gained (15 kg), average daily gain (0.155 kg/d), and hot carcass weight (10.86 kg) linearly increased with the length of time animals were fed during the 98 day finishing phase. Additionally, dry matter intake decreased by 0.616 kg/d and therefore G:F increased by 0.015 when the tannin blend was fed 68 and 100% of the time compared to animals that were not fed the tannin blend.

A study looking at the influence of feeding 28.14 g/hd/d of a condensed and hydrozable tannin extract blend continuously in ground corn-based diets to finishing bulls during a 226 day finishing trial (Barajas et al., 2011b) resulted in an increase in final body weight by nearly 37 kg and an increase in average daily gain of 0.162 kg/d. On days 28, 56, and 161 plasma urea nitrogen was also measured. On days 28 and 56 plasma urea nitrogen samples were collected and pooled, a decrease of 1.48 mg/dL was seen in cattle fed the tannin blend. These results were also observed on day 161. Because of the decreased level of plasma urea nitrogen found in the blood of the cattle fed the tannin

supplement, it can be speculated that protein digestion and absorption may be diverted from the rumen to the small intestine while maintaining a positive effect on gain.

Camacho et al. (2011) observed that feeding a blend of condensed and hydrolysable tannins at an inclusion rate of 0.32% of DM positively affected final body weight (36.8 kg greater than control) as well as hot carcass weight (25.43 kg greater than control) and rib eye area (3.45 cm²) when added to bull calf diets on a ground corn- based diet. Inclusion of the tannin blend however did not seem to effect dressing percentage, back fat thickness, kidney, pelvic, and heart fat, or marbling score.

Adverse to the previously listed studies, Krueger et al. (2010) found no effects on steer performance or feed efficiency when two different types of tannins were compared to a control diet, one derived from chestnut (a hydrolysable tannins) and the other from mimosa trees (a condensed tannin), were included in finishing steer diets at 14.9 g/kg of dry matter during a 42 day finishing period. Additionally, authors observed a reduction in HCW (averaging 9.3 kg between treatments and control) and empty body weight (averaging 10.3 kg between treatments and control) with tannin inclusion, with rumen and empty GIT being significantly heavier (averaging = 1.3 and 0.9 g/kg EBW, respectively) in cattle fed the chestnut tannins than those fed mimosa tannins or the control diet. The investigators speculated that the conservative level of tannin included in these diets could have been a contributing factor to the lack of significant positive results.

Effects of tannins on animal health

Tannins have also been studied regarding bactericidal and bacteriostatic effects as they are able to interact with bacterial cell membranes (Cannas, 2014). Studies have

showed effective action against *Escherichia Coli* (Min et al, 2008) and *Salmonella* Typhimurium (Costabile et al, 2011), as well as other strains of bacteria, making tannins a potentially valuable resource in food safety and a positive influence on animal health.

Costabile et al. (2011) studied the in vitro effects two different types of chestnut tannin concentrations, two concentrations of quebracho tannin, and one type of each tara, Chinese galls (sumach), and green tea tannin concentrations for their ability to inhibit the growth of *Salmonella typhimurium*. All these compounds have unique chemical structures. Authors observed that all types of tannin showed significant inhibition of growth rate, up to 1.28 log₁₀ reduction with gallotannins, on *Salmonella* but that dose was a factor having highest reduction rates at 3 mg/mL for chestnut wood at a 75.2% concentration, 6 mg/mL of 91.6% concentrated chestnut wood, 3 mg/mL for tara tannins (95.1% concentrate) and 1 mg/mL for sumach derived tannins (94.0% concentrate).

Min et al. (2008) tested *Escherichia coli*, *Klebsiella pneumoniae*, and *Staphylococcus aureus* against eight different types of condensed tannins purified from woody plants in low (0, 2,4,and 8 mg tannin/mL) and high (0, 50, 100 mg tannin/mL) doses. Authors observed that type of and dose of tannin as well as the type of bacteria effected the efficacy of the tannin to reduce the microbial growth of the bacteria. *S. aureus* was inhibited by all types of tannin at the 4 mg/mL dose but most notably by the tannins derived from Shinnery oak, Post oak, and Locust sources. *S. aureus* and *E. coli* growth were both inhibited with linear significance at the 4 mg/mL dose with catechin, ellagetannin, and tannic acid being effective. Fernandez-Miyakawa, Elizondo, and Mercado (2009) tested the efficacy of quebracho tannins, chestnut tannins and a blend of

the two types of tannin on reducing the amount of clostridium perfringens bacterial activity in an in vitro study. They found that when compared to control, both the quebracho and chestnut derived tannins showed bacteriostatic effects while only the chestnut showed bactericidal activity against *C. perfringens* and that combined, the two showed greater bactericidal and bacteriostatic effects than the chestnut alone.

Evaluating cattle dairy cattle fecal matter, Langer (2013) observed that when condensed tannins were tested in an in vitro system there was a significant inhibition of growth on the gram positive bacteria present in the feces. It did not seem to have an effect on the gram negative bacteria present, notably having no effect on the inhibition of *E. coli*. This study shows some possibility for the use of tannins as an antibacterial application to help control flies and other unwelcome health issues resulting from bacterial growth when applied to feces.

Hoste et al. (2006) has shown that condensed tannins have a large effect on gastrointestinal nematodes. Sanfoin, sulla, and some trefoil types have shown to be most effective in this when tannins are between 4 and 8% of dietary dry matter. Although there seems to be some variability associated with species, age of plant, and part of plant. In vitro and in vivo studies by Brunet et al. (2007) demonstrated that condensed tannin extract of several monomers had a large effect on reduction of gastrointestinal nematode development.

It has also been noted that tannin inclusion can decrease bloat while increasing animal efficiency and productivity in many situations such as planting a highly digestible legume with a higher tannin content in a pasture situation, such as birdsfoot trefoil

(MacAdam et al., 2013). However, the amount of tannin in the forage necessary to prevent bloat is not precisely known though Li and Larkin (1996) has suggested that 5 g of condensed tannin/kg of DM or more is adequate to prevent bloat without causing any detrimental effects to protein absorption, simply precipitating proteins from the rumen to a later site of absorption.

Effects of tannins on meat quality

Antioxidants have the power to prolong shelf life of meats. An antioxidant can vary in chemical structure but are often present in plants. An antioxidant is classified as a substance slows down oxidizing chain reactions (Velioglu et al., 1998). These reactions are what causes food spoilage. Antioxidants present in plants include, but are not limited to, vitamin C or ascorbic acid, vitamin E or alpha-tocopherol, the vitamin A precursor beta-carotene, many flavanoids, as well as other phenolic compounds (Pennington and Fisher, 2009), which would include tannins as they are polyphenolic and or flavenoid structures. Color is a very important factor for consumers when selecting a meat product as they associate color to freshness (Velasco and Williams, 2011). Oxidation of oxymyoglobin, which is red in color, to metmyoglobin, which is more brown in color, is main reason meat loses its appeal to consumers (Nerin et al., 2006). Refrigerated meat is especially susceptible to lipid oxidation because of its increased surface area, but adding an antioxidant pre harvest, as in to the animal's diet, or post harvest, as in on the surface of the meat or into the packaging, can help prolong shelf life (Velasco and Williams, 2011).

Gallic acid, a product of tannin digestion in ruminants, is known to have a high antioxidant capacity, especially for a natural compound (Velasco and Williams, 2011). Therefore adding tannins to diets of ruminant animals may have some effects on meat quality. However, adding an antioxidant to meat, either in the diet of the animal it came from or the during processing and packaging, may change the palatability factors that consumers value such as flavor, juiciness, and tenderness and so producers and processors must be aware of such potential negative impacts when selecting an antioxidant.

Zembayashy et al. (1999) fed tea leaves to Japanese heifers to examine their effect on meat quality and composition. They found that the heifers had a reduced muscle iron content. A strong negative correlation between the content of iron in the muscle and the lightness of the muscle was established. The tea leaves generally have a very high tannin content, in this case catechins, and it was concluded that the amount of tannin fed had a direct impact on the lightness of the meat. However, they were unsure of the mechanism in which the tannin effected the meat color.

Garg et al. (1992) reported that when cattle were fed tannins derived from *Quercus incana* leaves, a type of oak tree, no differences in blood iron existed even when blood hemoglobin levels were altered by tannin poisoning. They suggested that presence of tannins in the diet to not change iron absorption but possibly change the way the body utilizes the iron for synthesis of hemoglobin.

Based on this research, we hypothesize that inclusion of a tannic acid blend (ByPro®) will increase animal growth performance, digestibility of nutrients, reduce

fecal nitrogen volatilization, and increase shelf life of retail meat through a reduction
lipid oxidation.

CHAPTER III

MATERIALS AND METHODS

All procedures involving live animals were approved by the Texas Tech University Animal Care and Use Committee (Protocol # 13033-04)

Cattle receiving and processing

One hundred sixty-two (162) medium-large framed commercial beef steers (British x Continental) were received on May 23-24, 2013 at the Burnet Center, Texas Tech University Research Farm, near Idalou, Texas. Upon arrival, animals were randomly allocated into receiving pens (n = 14) with 12-15 animals per pen. On May 27, 2013 each steer received a numbered ear tag; treated with internal and external parasiticide (Dectomax, doramectin, Lot #OBXMF, exp. 12/2016); vaccinated with Bovishield Gold 4 (Pfizer, Lot#1280111, exp. 07/2014); metaphylactically treated with tilmicosin (Micotill 300, Lot# A877883/A917443, exp. 09/2014); and individual BW measurements were taken. On May 31, 2013 cattle were again processed, receiving a clostridial and pasteurilla vaccine (One Shot Ultra 7, Pfizer, Lot#1288437, exp. 04/Feb/2014; Lot #1289474, exp. 18/Jan/2014); treated for internal parasites (Safe-Guard- febendazole 10% Intervet/Scheuring-Plough, Lot# E070A01, exp. 12/2013); implanted (Component TE-IS, Elanco, Lot#02312T, exp. Nov/2015); and individually weighed. Based on this second unshrunk BW measurement, 18 steers, lightest/heaviest, bad temperament and physical or health issues were eliminated from the study pool. The remaining 144 steers were then used for BW blocks (n = 12) assignment. Extra steers not being used in the experiment were cared for in accordance with the Standard Operating Procedures at the Burnett Center. On June 3, 2013 steers were sorted into pre-establish

BW blocks, then returned to receiving pens. The following morning (June, 4 2013), steers within each BW block were stratified by BW and assigned randomly within strata to 1 of 3 pens (4 steers/pen - concrete, partially slotted floor; 3 m wide x 6 m deep; linear bunk space = 250 cm). Dietary treatments were then randomly assigned to pens within each BW block. Steers were kept under observation with same dietary regimen for another 5 days. On June 10, 2013, initial BW measurements were taken (study beginning). Until this date, steers were kept limit-fed (1.5% BW) on a receiving 65% concentrate diet (Table 1). Prairie hay (0.5 kg/steer/day) was top fed only during the initial 5 days after arrival. Immediately after initial BW measurement, the step-up 2 diets (75% concentrate) were fed, followed by step-up 3 diets (85% concentrate), until finisher diets being fed (92% concentrate). Step-up diets 2 and 3 already contained the respective tannic acid (ByPro®) inclusion, and were fed during 4 days each (Table 1). A common mineral and vitamin supplement (Table 2) balanced for the finishing diets was also included to receiving and adaptation diets.

Experimental design and dietary treatments

The three dietary treatments were arranged in a randomized complete block design. Pen (4 steers) was the experimental unit, with 1 pen per treatment inside each BW block (n = 12), resulting in 12 pens per treatment, and total 36 experimental units evaluated. Ingredients and dietary analyzed nutritional composition is shown on Table 3. Treatments consist of: (CONTROL) no tannic acid (premix added to the diet contained only carrier – cottonseed meal); (30 ByPro®) tannic acid to provide and estimated intake of 30g DM of ByPro®/steer/day; and (60 ByPro®) tannic acid in the diet to provide an

estimated intake of 60g DM of ByPro®/steer/day. Dietary treatments were based off a series of research trials by Barajas et al (2011a, 2011b, 2012a, 2012b) using only a 30 g/animal/day dose of ByPro®. Each diet contained the same mineral and vitamin supplement (Table 2) to meet or exceed NRC (1996) requirements, monensin (30 g/ton DM basis), and tylosin (9 g/ton DM basis). The percentage of premixes included in the diets was determined by assuming an average of DM intake per animal daily of 9.1 kg. Tannic acid product (ByPro®) premix mixtures were prepared on average for batches to supply needs of 15-20 days. The desired intake of ByPro® (30 and 60 g of DM/animal daily, respectively) was divided by the assumed DM intake of 9,070 g to calculate the percentage inclusion of ByPro® in dietary premixes (Table 1; Table 3).

Management, feeding, and weighing procedures

Standard procedures at the Burnett Center were used throughout the study. All low moisture-ingredient from diets were mixed in a 45- cubic foot capacity Marion paddle mixer. The Burnett Center feed milling system is operated by a computer-controlled WEM batching system. Once a total amount of low-moisture ingredients for a giving treatment was mixed, the feed was conveyed to a Rotomix 84-8 self-propelled mixer/delivery wagon, where the high-moisture ingredient (wet corn gluten feed) was mixed.

Diet samples were obtained on a weekly basis for all the treatment diets. Samples were collected as feed was discharged into the bunk by the Roto-Mix 84-8 unit, or taken directly from the feed bunk soon after feed has been delivered. Sub-samples from the same dietary treatment were composited to an approximately 400 to 500 g sample. One

portion of the sample was stored frozen for subsequent determination of the chemical composition (dried at 60°C for 48 h, force-air dry oven), whereas another portion was dried at 100°C for 24 h (forced-air dry oven) to determine the dry matter content of the diet. Frozen samples were composited by treatment diet for each 35-d period of the study. Samples (60°C) were ground to pass a 1 mm screen in a Wiley mill, and overall composites were analyzed for DM, ash, CP, NDF, ADF, Ca, P, K, S and ether extract (Table 3) in a commercial laboratory (ServiTech Labs, Amarillo, TX), with the exception of crude protein, which was analyzed by Kjeldhal method (AOAC, 1995) in house.

Feed bunks were inspected visually (read) once daily approximately 0700 to 0800 h. Feed was allotted to pens so that 0 to 0.5 kg of feed was left in the bunk at the time of bunk reading. All cattle were fed once per day in the morning. At the time of bunk reading, a computer-generated feed bunk reading sheet was used to determine what each pen received during the previous 3 days, so that the amount to feed the pen for the current day can be based on the previous pattern of consumption and any feed remaining in the bunk. Increases of the feed allotted to a pen by 0.15 to 0.20 kg per animal daily, with the increase not exceed 0.25 kg per animal daily. When the feed allotted to a pen needed to be decreased, the decrease was based on a visual estimate of feed remaining in the bunk at the time of the bunk reading. When feed was decreased to a pen, the bunk reading process on the following day took into account the feed offered on the previous day plus the estimated feed remaining in the bunk on the previous day. The weekly dietary DM values (100°C) during a weigh period were used in conjunction with feed bunk weigh-back samples to determine the feed intake by the animals in each pen.

Intermediate BW measurements were obtained on a pen basis at 35-d intervals during the study. Initial BW (individual) was taken on day 0 (June 10, 2013). Pen weights were taken on d-35 (July 15, 2013), d-105 (September 23, 2013), and d-140 (October 28, 2013). Individual animal weights were taken on d-70 (August 19, 2013) during cattle re-processing, and at harvest shipping moment to establish a full final BW for each animal. On d-70 (re-processing), steers were re-implanted with Component TE-S (Elanco Animal Health, Lot# 10213T, exp. June/2015); poured with Ultraboss (permethrin, 3mL/100 lb, Lot #97008C). All scales were validated with at least 500 kg of certified weights before use.

Harvesting and carcass measurements

Body weight blocks were shipped to a commercial harvesting facility (Tyson Fresh Meats in Amarillo, TX) when approximately 60% or greater animals in a given block had sufficient finishing to grade USDA Choice. The heaviest 3 blocks of steers were shipped after 121 days on feed, the next 5 heaviest blocks after 156 days on feed, and the remaining 4 lightest blocks after 175 days on feed. Personnel from West Texas A&M University collected HCW and liver abscess data, and the in-plant camera system was used to determine yield grade, quality grade, marbling score, LM area, and fat thickness at the 12th rib. Liver condemnations were classified for liver abscesses using methods described by Brink et al. (1990). Dressing percent was calculated by dividing HCW by non-shrunk final BW. Carcass-adjusted BW was calculated from HCW divided by the average DP across treatments (62.14%) and adjusted by a 4% shrink. Carcass-adjusted ADG was calculated from carcass-adjusted final shrunk BW, initial BW, and

days on feed, and carcass-adjusted G:F was calculated as carcass-adjusted ADG divided by average DMI for the experimental period.

Carcasses from the first (n = 32) and second (n = 30) harvest group grading USDA Select and Choice (n = 6 Select *harvest group 2 only*; n = 56 Choice) were identified by personal from the West Texas A&M Beef Carcass Research Center. One trip loin from each carcass was tagged and collected during fabrication approximately 48 hrs after harvest. Following fabrication strip loins were individually vacuum packaged and boxed for shipment to Texas Tech University. Strip loins were maintained at 2-4°C until 7 days post mortem when they were further processed.

Apparent digestibility evaluation

Starting on d-90, a five day digestion phase was conducted. Samples of the experimental diets and bunk refusals were collected daily from d 90 through 96. Two samples were taken, 1 for analysis of DM, and the other was frozen for later chemical analysis. Fecal samples from at least 2 animals per pen (usually collected from all 4 animals) were taken from the concrete pen floor immediately after defecation. Collection of fecal samples occurred twice daily at approximately 0800 and 1600 h on d 92 through 96. Fecal samples were frozen and stored individually after each collection day. On the last day of collection, samples were sorted by pen, day of collection and thawed overnight. The next morning, samples were homogenized inside each collection bag then composited by pen (100 ± 0.5 g of wet basis) generating the total of 36 samples. Composite fecal samples (500g) were forced-air oven dried at 55°C for 72 h. Feed (composited by treatment; n = 3) and feed refusals (composited by pen, n = 36) were

dried at 55°C for 24 hrs. Feed, refusals, and fecal samples were ground in a Wiley mill (Thomas Scientific, Swedesboro, NJ) to pass a 1-mm screen. Daily refusals from digestibility evaluation were composited by percentage of weight (10%) recovered from bunk from each morning before feeding, homogenized then sub-sampled following procedures already described. Acid insoluble ash was used as internal marker to estimate total fecal output and consequent apparent digestibility coefficients for nutrients (Van Keulen and Young, 1977). Apparent total tract digestion of DM, OM, CP, and starch were calculated as follows: $100 - 100 \times [(AIA \text{ concentration in feed}/AIA \text{ concentration in feces}) \times (\text{nutrient concentration in feces}/\text{nutrient concentration in feed})]$. Nutrient concentrations were corrected for orts (i.e., orts-corrected quantity of nutrient consumed divided by orts-corrected quantity of DM consumed)

Fecal Nitrogen Volatilization

After animals were shipped, feces in pens was allowed to air dry for 7 days. 500 g of feces were then collected from the driest surface areas of the pens. Samples were ground in a Wiley mill to fit through a 1 mm screen then stored for further analysis. Fecal nitrogen volatilization was calculated as by Cole et al. (2006) and is as follows:

$$\text{N Volatilization (\% of intake)} = (\text{N:P of diet} - \text{N:P of manure})/(\text{N:P of diet}).$$

$$\text{N Volatilization (g/day)} = ((\text{N volatilization as \% of intake})/100) * \text{N intake (g/day)}.$$

$$\text{N Volatilization (kg/steer)} = ((\text{N volatilization g/st/d}) * \text{DOF})/1000$$

Laboratory analyses of diets, refusals, feces and pen surface samples

Diets composited by period were sent to a commercial laboratory (Servitech Labs, Amarillo, TX) for chemical analysis. Results are displayed in Table 3. Samples of diets,

orts, and feces collected during the digestion and were analyzed for acid insoluble ash, total starch, N, OM, NDF, and P. Pen air-dry surface fecal samples from N volatilization phase were analyzed only for N and P. Total starch and P content was analyzed by a commercial laboratory (Servitech Labs, Amarillo, TX). For N determination, approximately $0.5 \text{ g} \pm 0.005 \text{ g}$ of ground feed and orts, and $0.3 \text{ g} \pm 0.005 \text{ g}$ of fecal samples were analyzed using the combustion method (Leco, Model: TruMac N, St Joseph, MI; Method 4.2.10, AOAC, 1997). Ash content was determined by burning each sample in a muffle furnace at 600°C for 4 h and used to determine OM. Acid-insoluble ash was analyzed as described by Van Keulen and Young (1977) using the 2N HCl method and is as follows: 5 g of sample $\pm 0.005 \text{ g}$ of feed, feces, and orts were weighed and dried for 2 hours at 135°C in a pre-weighed crucible. They were weighed again after drying to obtain a dry matter then ashed at 450°C overnight. Ashes were then combined with 100 mL of 2N HCl in a 200mL beaker and boiled for 5 minutes on a hot plate under a fume hood. Next, contents were rinsed with 100°C deionized water and filtered onto ashless filter paper. Filters were then put into a second pre-weighed crucible and ashed overnight at 450°C and weighed to obtain the amount of acid insoluble ash remaining.

NDF was determined using the ANKOM Technology Method 6 Neutral Detergent Fiber in Feeds Filter Bag Technique for A200, A2001, A220 models. ANKOM F57 filter bags were individually numbered with an industrial strength sharpie and weighed. $0.5 \text{ g} \pm 0.005 \text{ g}$ of ground feed, orts, and feces were weighed and put into the pre-weighed filter bags. Bags were then heat sealed and placed on ANKOM shelving. An empty bag was included in every sample run for correction. 1 L of neutral detergent

solution and 20g of sodium sulfite were added to the ANKOM apparatus, the shelving was placed in the apparatus, and another 1 L of neutral detergent solution was added as well as 4 mL of alpha-amylase. The samples were then digested for an hour at 100°C with constant agitation. After 1 hour at 100°C, solution was drained and samples were rinsed 3 times with 2 liters of 100°C deionized water for 5 minutes each rinse, the first two rinses containing 4 mL of alpha-amylase. Next bags were soaked in acetone for 5 minutes before being dried at 100°C for at least 4 hours. After drying, bags were reweighed and ashed to correct for ash content.

Meat analyses

Proximate Composition: At the time of subprimal processing, the anterior portion (approximately first anterior 2 inches) of each subprimal was reserved for analysis of proximate composition. Samples were individually packaged and frozen (-20°C) until later analysis. Prior to analysis, sections were thawed, trimmed of external fat, and ground using a commercially available grinder (Kitchen Aid with grinder adapter, Model KP26M1XER Professional 600, USA) to obtain approximately 200 g of sample. Compositional analysis of moisture, fat, protein, and collagen was conducted using an AOAC-approved (Official Method 2007.04; Anderson, 2007) near-infrared spectrophotometer (FOSS Food Scan™ 78800; Laurel, MD).

Raw color analysis. Following removal of a section for proximate analysis, a 2.54-cm thick steak was removed for evaluation of retail display color. Single steaks were placed on a black expanded polystyrene trays (Cryovac Ltd., Duncan, SC, USA). Trays were

overwrapped with PVC film (MAPAC L; oxygen transmission rate [OTR] = 21,700 cc of O₂ per m² per 24 h; Borden Packaging and Industrial Products, North Andover, MA, USA) using a heat-sealing overwrap machine (Heat Sealing Equipment Co., Cleveland, OH, USA). Immediately following packaging, trays were placed in coffin-style (Model H1, Hussman, Bridgeton, MO, USA) retail display cases under continuous fluorescent lighting (1,900 lux) using high-output bulbs with a color temperature rating of 3,500° Kelvin and a color rendering index of 70. Packages were kept in the retail case for 7 d at 0 to 2°C. Each d, for 7 d, instrument color (CIE L^* , a^* , and b^*) of strip steak packages was measured through the packaging film at three different locations across the package surface using a portable spectrophotometer (Hunter Miniscan XE Plus, Hunter Laboratories Model MSXP-4500C, Reston, VA, USA) with illuminant A, a standard observer angle of 10°, and 2.54-cm aperture (CIE, 1978). Instrument calibration was completed before use at each sampling period using black and white tiles covered with PVC packaging film (AMSA, 2012). CIE L^* , a^* , b^* values were used to calculate hue angle ($\tan^{-1} a^*/b^*$) and saturation index ($((a^2 + b^2)^{1/2})$) for each sample. The observations ($n = 3$) were averaged prior to statistical analysis.

Additionally, reflectance for calculation of relative pigment proportions (% oxymyoglobin, deoxymyoglobin, and metmyoglobin) were obtained using methods developed by Kryzwicki (1979) as described by AMSA (2012). Briefly, the portable spectrophotometer described previously was utilized to measure reflectance at 470, 525, 575, 580, 610, 630, 680, 700, and 730 nm on three different locations on the loaf. The reflex attenuation of each wavelength was obtained by calculating the logarithm of the

reciprocal of the reflectance. The reflex attenuation for 470, 525, 575, and 730 were used to calculate the relative proportion (%) of metmyoglobin, deoxymyoglobin, and oxymyoglobin using formulas summarized in AMSA (2012).

Furthermore, a discoloration/redness ratio was calculated from the reflectance values at 580 and 630 nm as described by AMSA (2012). This ratio is indicative of the relative proportion of oxymyoglobin, the predominant pigment at 630nm, to metmyoglobin, which is predominant at 580nm. Thus, values closer to 1.0 are indicative of discoloration.

Lipid oxidation. Samples for evaluation of thiobarbituric acid reactive substances (TBARs) were obtained at the time of subprimal portioning. At portioning, a sample was obtained from the anterior end of the subprimal, frozen in liquid nitrogen, placed in an individually labeled Whirlpak[®] sample bag, and stored (-80°C) until later analysis. Immediately prior to analysis, samples were homogenized using a commercial blender and liquid nitrogen. Approximately 10 g of the homogenate was added to a 50 ml centrifuge tube and analysis of TBARs proceeded as described by Luque et al. (2011). Samples were evaluated in duplicate and averaged prior to statistical analysis.

Statistical analyses

Performance data were analyzed with pen as the experimental unit in a randomized block design using the GLIMMIX procedures of SAS (SAS Inst., Inc., Cary, NC). Model included the fix effect of treatment and random effect of block. Least square mean differences were adjusted by test of Tukey, and bias of degrees of freedom adjusted by Kenward-Roger degrees of freedom method. Carcass data (USDA Quality Grade and

liver scores) were entered on an individual animal basis, with model including the same parameters as described previously. Due to the nature of carcass data distribution (non-Gaussian), the Link function of GLIMMIX procedure of SAS was used for data analysis of treatment effects, and the inverse-Link function was used for reporting of responses. Pre-planned linear and quadric effects of ByPro® inclusion (0, 30 and 60 g DM of ByPro®/animal/day) contrasts were evaluated. Data from evaluations of meat quality traits were analyzed using a linear mixed model (PROC MIXED). ByPro® treatments, retail display day (where appropriate), and their interaction served as independent variables of interest. Harvest group ($n = 2$) was utilized as random variable while carcass QG was incorporated as a covariate. Least square means, generated for independent variables and their interaction (where appropriate), were separated using the PDIFF option and differences considered significant at $P < 0.05$.

CHAPTER IV

RESULTS AND DISCUSSION

Cattle performance

Intake, gain and gain: feed ratio (G:F) are shown in Table 4. Cumulative performance data are shown only d 0 to 35, d 0 to 70, d 0 to 105 and d 0 to 140 because of the different days on feed for the three harvesting groups after day 121. Data labeled day 0 to end in Table 4 represent the average from the beginning to the end of the study for all respective harvesting blocks of steers (heavy block = 121 days on feed and light block = 175). Even though the first 3 body weight blocks were shipped on day 121, the remaining 9 blocks were analyzed until day 140 for cumulative data. Beyond day 140, data was not included in cumulative evaluation because only few blocks ($n = 4$) remained to be analyzed.

Intake quadratically increased ($P = 0.05$) from d 0 to 35 with ByPro® inclusion. Intake from d 0 to 105 ($P = 0.07$) and d 0 to end tended ($P = 0.06$) to increase linearly, with DMI of steers consuming 60-ByPro® being 3.7% greater than CON. No differences on DMI were observed during 0-70 ($P = 0.34$) and 0-140 ($P = 0.63$) with intake averages being 9.74 and 9.94 kg/d respectively. No differences were observed for ADG during the current study ($P \geq 0.16$). The ADG for days 0 -35, 0-70, 0-105, 0-140, and 0-end averaged 2.11, 1.87, 1.90, 1.86, and 1.64 kg, respectively. Although not statistically different, steers fed ByPro® showed approximately 80g/d ($P = 0.16$) and 50g/d ($P = 0.17$) linear increase of gain, during days 0-70 and 0-105, respectively. ByPro® inclusion did not affect overall (d 0 to end) carcass-adjusted ADG ($P = 0.65$) or G:F ($P \geq 0.17$),

averaging 1.62; 1.66; and 1.64 kg/d, and 0.163; 0.163; and 0.159 kg gain/kg of DMI, for CONTROL, 30-ByPro®, and 60-ByPro®, respectively. The G:F for days 0-35 ($P = 0.95$), 0-70 ($P = 0.72$), 0-105 ($P = 0.78$), 0-140 ($P = 0.94$), and 0-end ($P = 0.58$) averaged 0.232, 0.192, 0.192, 0.187, 0.162 kg of gain/ kg of DMI, respectively. With few numerical differences, ADG did not follow the same findings as Barajas et al. (2012a). Cattle from current study were castrated and implanted while Barajas et al. (2012a) fed intact males. Lower roughage concentration (8 vs. 11.3%) and the use of steam-flaked corn for the current study instead of ground sorghum, may also played a role in the distinct results pattern. A longer feeding period (156 vs. 56 d) and overall heavier final body weight (600 vs. 456 kg) were also observed in the current study compared to Barajas et al. (2012a). Additionally, the sorghum grain fed in the study by Barajas et al. (2012) could have impacted tannin concentration, as sorghums can have very high inclusion levels of tannin. Based on the dry matter intake of bulls in the Barajas et al. (2012a) study, bulls could have been consuming as much as 10.2 g/kg of DM more tannin a day than they estimated.

The findings in the current study also conflicted with the findings of another study done by Barajas et al. (2012b) where yearling bulls were fed dry-ground corn based diets with a tannin level of inclusion at 0.33% of dietary DM where an increase in ADG of 0.187 kg/d was observed. Bulls fed the tannin blend also finished at 14.73 kg greater than the control. In contrast with our study, Barajas et al. (2012b) fed bulls for a finishing period totaling 84 days while the current study fed steers an average of 156 days. Diets also differed in content; Barajas et al. (2012b) fed a ground corn based diet, with an

average of 36.6% inclusion of ground corn over the average of the trial. Additionally, for the first 21 days, an average of 27.7% of corn silage was included. Barajas et al. (2012b) also used only true protein sources (canola meal and dry distillers grains), with no urea added. They also included a large amount of dry distillers grains (average of 12.59%) and a high inclusion of corn straw as a roughage source (average of 11.09%). The findings of the current study are similar to those of this study as no difference in DMI was observed over the feeding period. The major difference in roughage concentration, protein source, and length of feeding period between the current study and the study by Barajas et al. (2012b) could all influence the differing results observed by researchers.

A study by Barajas et al. (2011a) observing length of inclusion of blended of hydrolysable and condensed tannin (ByPro®) on performance of finishing bulls found that feeding bulls a 96% concentrate, dry distillers grain-ground corn based diet for 0, 68, and 100% of 102 days showed an increase in final BW (14.95 kg) for cattle fed the entire finishing phase. Cattle in this study by Barajas et al. (2011a) were *Bos indicus x Bos Taurus* bulls and were fed in Mexico. There were 20 bulls in each treatment group (avg . initial BW = 366 kg), separated into pens of 5. Additionally, authors observed total weight gain of 15 kg, and ADG of 0.155 kg/d, and HCW of 10.86 kg greater for cattle fed the longest amount of time compared to control. Dry matter intake decreased by 0.616 kg/d and therefore G:F increased by 0.015 when the tannin blend was fed 68 and 100% of the time compared to animals that were not fed the tannin blend, while DMI in the current study were not affected. Although similar amounts of inclusion of the same tannin blend were observed (0.33% of dietary DM for Barajas et al. (2011a) and 30-ByPro® in

current study), the current study is once again in contrast with this study in diet, days on feed, and use of growth technology (implants and ionophores). Additionally, Barajas et al. (2011a) included only 12.6% CP all from true protein sources while diets in the current study averaged 13.4% CP, with a portion of that coming from a NPN source.

A study looking at the influence of feeding 28.14 g/hd/d of a condensed and hydrozable tannin extract blend (ByPro) continuously in ground corn-based diets to finishing bulls (*Bos indicus* x *Bos taurus*, avg IBW = 184 kg) during a 226 day finishing trial (Barajas et al., 2011b) resulted in an increase in final body weight by nearly 37 kg and an increase in average daily gain of 0.162 kg/d. Diets in this study contained 37.68% corn straw for the first 28 days, 30.42% corn straw from days 29-105, 18.05% corn straw from days 106-133, and 14.02% corn straw from days 134 to the end of the trial. Whereas steers fed in the current trail received 12.50% inclusion of both alfalfa hay and cottonseed hulls for the first 4 days of the finishing period, 7.50% alfalfa and cottonseed inclusion for days 5-8, and then only 4.0% inclusion of both alfalfa and cottonseed hulls for the remainder of the feeding trail. This major difference in roughage concentration between this study and the current study can offer some explanation to the differences seen in performance. ADG for this trail was 1.365 and 1.527 kg/d for control and treatment, respectively. For the current trial, ADG was 1.62, 1.66, and 1.64 kg/d for control, 30-ByPro®, and 60-ByPro®, respectively. The control cattle in the current experiment gained an average of 0.25 kg/d greater than control cattle in this study. Additionally, cattle receiving the same amount of ByPro® (treatment cattle and 30-ByPro®) in the current study as in this study gained 0.13 kg/d more. Again, difference that may explain

these contrasts between the current study and this study exist in castration status, diet, protein source, use of growth technologies, and length of feeding.

These differences coupled with greater DMI observed for steers fed ByPro® during the first half of the current study may suggest a greater potential for use in increased production of ByPro® in earlier stages of the feeding phase. Despite the greater intake and only trivial numerically greater gain until day 105, the feed efficiency for steers fed ByPro® was not negatively impacted in the current study. This might be reflecting the opportunity of using tannic acid (ByPro®) during earlier strategic moments of animal growth and diets. This hypothesis is supported by the fact that positive gain effects of ByPro® on bulls were observed when animals were harvested earlier as far as body weight and days on feed compared to the current evaluation (Barajas et al, 2011 a,b; Barajas et al. 2012a,b).

Carcass characteristics

Carcass measurements are shown in Table 5. Carcass characteristics including, HCW (388 kg; $P = 0.52$), fat thickness (1.47 cm; $P = 0.32$), longissimus muscle area (94 cm²; $P = 0.57$), and yield grade (3; $P = 0.29$) were not affected by dietary inclusion of ByPro. No differences in quality grade ($P = 0.44$) were observed between treatments with premium choice, choice, upper choice, and select averaging 35.7, 51.0, 88.1, and 11.9 %, respectively. Despite slight numerical differences, ByPro® did not affect dressing percentage (quad; $P = 0.19$), but tended to quadratically increase KPH ($P = 0.12$) by approximately 1 percentage unit for steers fed ByPro-30® compared to control. A quadratic decrease trend was observed in the percentage of liver scores taken for total

number of liver scores ($P = 0.13$) and (A) scores ($P = 0.17$) for steers fed ByPro®. No differences were observed in the percentage of livers scoring A+ ($P = 0.88$) with overall average being 4.91%. However, while not being statistically significant, control total liver scores were 8.67% greater than the average of the two ByPro® treatments (overall average = 8.36%)

Camacho et al. (2011) observed that feeding a blend of condensed and hydrolysable tannin extract at an inclusion rate of 0.32% of DM to *Bos indicus* x *Bos Taurus* bull calves (n=20, IBW = 184 kg) positively affected final body weight at 36.8 kg greater than control. Hot carcass weight (25.43 kg greater than control) and rib eye area (3.45 cm² greater than control) were also positively influenced when the tannin blend was added to bull calf diets on a ground corn- based diet. These findings are in contrast with the current study. Similar to the current study, Camacho et al. (2011) observed that inclusion of the tannin blend however did not seem to effect dressing percentage, back fat thickness, kidney, pelvic, and heart fat, or marbling score. Diets fed by Camacho et al. (2011) were dry-ground corn, canola meal, and dry distiller grain based which are different from the steam-flaked corn based diets with wet corn gluten feed that were fed in the current study. Additionally, no non-protein nitrogen sources were fed in this study but were included in the current study. A difference in final body weight is also evident between the two trials. Steers from the current study were harvested at an average of 599.3 kg while the final body weight of the bulls in this study averaged 510.8 kg, a difference of 88.5 kg of total BW. HCW was 62.1 kg greater in the current study than observed by Camacho et al. (2011). Another difference between studies is noted in the

marbling scores of the cattle. Based on the scales used in the two separate trials, bulls fed by Camacho et al. (2011) had a marbling score just below small (average = 465 with 400 = Light and 500 = Small), while steers in the current study averaged just below a moderate amount of marbling (average = 48.28 with 40 = small and 50 = modest). There was a difference in KPH of 0.14 percentage units between trails, averaging 1.91 and 2.05% between this study and the current study. LM area was also 12.08 cm² greater for steers in the current study than bulls in this study. Diets between this study and the current study also differed. Bull-calves in this study were fed a step up diet that contained 37.68% corn straw for the first 28 days, 30.42% corn straw from days 29-105, 18.05% corn straw from days 106-133, and 14.02% corn straw from days 134 to the end of the trial. Whereas steers fed in the current trail received 12.50% inclusion of both alfalfa hay and cottonseed hulls for the first 4 days of the finishing period, 7.50% alfalfa and cottonseed inclusion for days 5-8, and then only 4.0% inclusion of both alfalfa and cottonseed hulls for the remainder of the feeding trail. This difference in roughage concentration and length of high inclusion of roughage concentration could have had an effect on carcass characteristics.

A study by Kruger et al. (2010) saw a 9.3 kg difference in HCW between steers fed either chestnut or mimosa tannins while there was no difference in the current study between treatments on HCW. Similar to Kruger et al. (2010), no other significant differences in carcass characteristics between treatments were found. Differences in diets between the two studies exist, Kruger et al. (2011) feeding sorghum hay at an inclusion of 71.1 g/kg DM. As previously stated, sorghum potentially contains large amounts of

tannin. Tannin treatment was included at 14.9 g/kg DM, unlike the per head basis in the current study. The level of tannin included in the diets fed by Kruger et al. (2010) differed at least 108 g/hd/d of tannin inclusion based on the DMI of the cattle in this study (average treatment diet = 11.33 kg DMI x 14.9 g/kg DM = 168 g/hd/d) vs the 60-ByPro® (60 g/hd/d) treatment in the current study. This large addition of tannin to the diet could explain the difference in HCW based on feed efficiency and possible tannin toxicity experienced by cattle in this study. Additionally, cattle fed by Kruger et al. (2010) had a HCW averaging 280 kg while cattle in the current study had an average HCW of 388 kg, 108 kg of carcass weight difference. Yield grade between the two studies differed by only 0.25, with the current study being greater. Marbling score also differed between the two trails, steers fed by Kruger et al. (2010) having an average marbling score closer to small (average = 4.91 with Slight = 4.00 and Small = 5.00) while the current study observed a much more desirable marbling score (average = 48.28 with 40 = small and 50 = modest).

Nutrient intake and digestibility

Dietary coefficients of digestibility percentage of DM, OM, Starch, CP, and NDF are shown in Table 6 along with fecal output and nutrient composition of the diet for the 5-d digestibility evaluation. Acid insoluble ash (AIA) concentrations in diets averaged 1.80, 1.69, and 1.84 % for control, 30-ByPro®, and 60-ByPro® respectively. AIA concentrations in feces for control, 30-ByPro®, and 60-ByPro® averaged 7.08, 7.13, and 7.06 % respectively. Intake of DM ($P = 0.07$), OM ($P = 0.08$), and NDF ($P = 0.17$) tended to increase linearly with ByPro® inclusion. No differences were seen in starch or

CP intake. A linear increase with ByPro® inclusion in fecal output of starch ($P = 0.03$) and CP ($P = 0.04$) was seen. Quadratic decreases were seen fecal output of DM ($P = 0.17$) and OM ($P = 0.13$). However NDF fecal output did not differ between control, 30-ByPro®, or 60-ByPro® treatments. Apparent total tract digestibility of control diet was similar to those observed in previous studies evaluating on apparent total tract digestibility of SFC-based beef cattle finishing diets containing similar amounts of WCGF inclusion (Sindt et al, 2003; Domby et al, 2013). Apparent total tract digestibility of tended to increased quadratically for DM ($P = 0.14$) and OM ($P = 0.09$). A significant linear decrease ($P = 0.03$) in starch digestibility was observed with ByPro® inclusion. A linear ($P = 0.09$) tendency for CP apparent total tract digestibility to decrease with ByPro® inclusion was also observed. Although not statistically significant (lin = 0.36, quad = 0.41), NDF digestibility was 5.80% and 4.52% greater for cattle fed 30-ByPro® and 60-ByPro®, respectively.

The decrease in CP digestibility are similar to findings of Jayanegara and Palupi (2010) who evaluated in vitro and in vivo results from 19 different studies, culminating in the finding that CPD decreased linearly with inclusion of tannin. Adversely, they observed a consistent decrease in OMD while a quadratic tendency was seen for an increase in OMD in the current study. Because starch was the only feed component that showed a significant decrease in digestibility but a tendency for CP digestibility to decrease existed, the possibility exists that the difference in CP digestion negatively affected the starch digestion. It could also be that tannins complexed with starch components and failed to release them in the abomasum and duodenum where pH is

much lower than the rumen. It has also been suggested that animals have the ability to develop a tolerance to tannins with prolonged exposure (van Soest, 1994). Digestibility may be influenced more at earlier stages of inclusion which would coincide with the increase in DMI during the beginning of the feeding period.

Fecal nitrogen volatilization

There were no differences observed in fecal nitrogen volatilization as percentage of intake, g/steer/d, and total kg/steer (Table 7). Volatilization of N in feces in this trial, both treatment and control groups, was similar to volatilized amounts observed in diets containing similar DIP levels (average 8.93% DIP in the current study vs. 8.15%) in steam-flaked corn-based finishing diets (Cole et al., 2006). Authors in the later, observed an increase in fecal nitrogen volatilization as percentage of CP from non-protein nitrogen sources increased. The level of NPN in current evaluation was similar to the highest inclusion of NPN in the study conducted by Cole et al. (2006). Therefore, it may be inferred that the amount of true protein vs. NPN has an effect on the ability of tannins to bind to protein structures and not to NPN sources. It may also be possible that the level of tannin included in the diets in this study is simply not high enough to create any sort of significant difference in its ability to complex with proteins completely and maintain that complex in feces.

Meat analyses

Historically, consumers have utilized lean color at retail as their primary indicator of meat freshness and exhibit a preference for a bright cherry-red lean color (McMillin, 2008). As such, the maintenance of desirable lean color, and the incorporation of

strategies that promote its stability, is advantageous to the meat industry. Overall, ByPro® supplementation had little influence on objective evaluations of the lean color of overwrap packaged beef strip loin steaks during retail display. Instrument-based assessment of lean color lightness (L^*), redness (a^*), yellowness (b^*), and hue did not differ ($P > 0.05$) among treatments (Table 9). However, saturation values, which are indicative of color depth and intensity, tended ($P = 0.07$) to be greater in steaks from cattle fed the Control or 30-ByPro® diets.

The bright cherry-red lean color, which consumers desire, is a result of oxymyoglobin formation (Mancini & Hunt, 2005). Conversely, consumers discriminate against the presence of brown or discolored lean, which is a result of the oxidation of oxymyoglobin to metmyoglobin. In the current trial, steaks from cattle fed the 60-ByPro® diet had a greater ($P = 0.002$) proportion of metmyoglobin than steaks from cattle fed control diet. These results correspond with the tendency for reduced saturation values, also indicative of discolored lean, in steaks from cattle fed the 60-ByPro® diet (Table 9).

ByPro® treatment and retail display day interacted ($P = 0.0017$, SEM = 3.40) to influence oxymyoglobin proportions during retail display (Table 10; Figure 1). All values regardless of treatment declined as display progressed. However, on day 5 control and 30-ByPro® treatments had lower proportions of oxymyoglobin than steaks from 60-ByPro®. The influence of retail display day on the objective color values and proportion of meat pigments are presented in Table 9. Retail display length did not have an influence on lean color lightness (L^* , $P = 0.18$); however, lean redness (a^*), yellowness (b^*), and

saturation values all decreased as display progressed. These results correspond with previous evaluations of beef lean color which have documented color deterioration in overwrapped beef steaks during retail display (Jeremiah & Gibson, 2001; Mancini & Hunt, 2005). Likewise, the proportion of metmyoglobin rose ($P < 0.0001$) as retail display progressed from 0 to 7 d. After 7 d of retail display, approximately 40% of the lean surface was in the metmyoglobin state.

ByPro® treatment and retail display length interacted to influence the proportion of oxymyoglobin present on the lean surface. As expected, oxymyoglobin proportions decreased during retail display for all treatments. No differences due to ByPro® diet were noted until days 5 through 7 of retail display when steaks from the 60-ByPro® and Control treatments exhibited greater oxymyoglobin proportions than steaks from the 30-ByPro® feeding groups.

As shown in Table 11, ByPro® inclusion into the diet had no influence on the pH ($P = 0.776$) or formation of lipid oxidation byproducts (as indicated by thiobarbituric acid reactive substances-TBARs) in beef strip loins aged for 21 d. Although dietary inclusion of ingredients with antioxidant activity has previously been shown to offer protection from lipid oxidation (Formanek et al., 2001; Georgantelis et al., 2007), no measureable protection from oxidation was observed in the current trial. These data support future investigations of dietary inclusion at greater levels than those employed in the current research.

TABLES AND FIGURES

Table 1. Dietary ingredients and calculated nutritional composition of receiving and step-up diets fed to steers.

Items	Adaptation diets		
	Receiving (65% conc.)	Step-up 2 (75% conc.)	Step-up 3 (85% conc.)
Days on feed	17	4	4
Period included in the study	No	yes	yes
<i>Ingredient inclusion, % DM</i>			
Steam-flaked corn (26 lb/bu)	38.72	47.32	57.05
Wet corn gluten feed, Cargill	20.00	20.00	20.00
Alfalfa hay	17.50	12.50	7.50
Cottonseed hulls	17.50	12.50	7.50
Yellow Grease	3.00	3.00	3.00
Supplement ¹	2.00	2.00	2.00
Urea	0.38	0.48	0.60
Limestone	0.90	1.20	1.35
Premix²	0	1.00	1.00
<i>Calculated Composition³, DM basis</i>			
DM, % as-is	82.1	80.13	79.73
CP	13.52	13.48	13.50
Ca	0.61	0.64	0.61
P	0.36	0.36	0.36
K	0.98	0.89	0.79
S	0.21	0.21	0.21
NEm, Mcal/kg	1.81	1.93	2.06
NEg, Mcal/kg	1.16	1.27	1.39

¹Supplement contained (DM basis): NaCl, 15%; CuSO₄, 0.12%; MnSO₄, 0.08%; Se Premix 0.2%, 0.25%; SnSO₄, 0.21%; Vitamin A, 550000 IU/kg; Vitamin E, 875 IU/kg; Rumensin (30 g/ton of monensin, DM basis, Elanco Animal Health, Indianapolis, IN); Tylan (9 g/ton of tylosin, DM basis, Elanco Animal Health).

²Premix contained: cottonseed meal, for control diet; 0.33% tannic acid (ByPro®) for 30-ByPro® diet; and 0.67% tannic acid (ByPro®) for 60-Bypro® diet. Cottonseed meal was used as carrier.

³Calculated composition based on regularly analyzed individual ingredients used in the Burnett Center Research Station.

Table 2. Composition of the TTU mineral and vitamin supplement used in experimental diets.

Items	%, DM basis
Cottonseed meal	67.7536
Kemin Endox	0.5000
Potassium Chloride	10.0000
Urea	3.7600
Salt	15.0000
Cobalt carbonate	0.0022
Copper sulfate	0.1965
Iron sulfate	0.0833
EDDI	0.0031
Manganous oxide	0.1667
Selenium premix, 0.2 %	0.1250
Zinc sulfate	0.9859
Vitamin A – 1,000,000 IU/g	0.0099
Vitamin E – 500	0.1575
Rumensin – 90 ^a	0.7500
Tylan – 40 ^a	0.5063
<i>Calculated composition (concentration in supplement)</i>	
CP, % DM	42.18
Ca, % DM	0.15
P, % DM	0.79
K, % DM	6.12
S, % DM	0.53
Mg, % DM	0.47
Co, mg/kg	10.36
Cu, mg/kg	511.18
Fe, mg/kg	419.87
I, mg/kg	25.00
Mn, mg/kg	1018.33
Se, mg/kg	3.16
Zn, mg/kg	3550.14
Na, mg/kg	6.05
Cl, mg/kg	13.83

^aRumensin (30 g/ton of monensin, DM basis, Elanco Animal Health, Indianapolis, IN); Tylan (9 g/ton of tylosin, DM basis, Elanco Animal Health, Indianapolis, IN).

Table 3. Dietary ingredient inclusion and analyzed nutritional composition of finishing diets containing levels of tannic acid (ByPro®) fed to steers.

Items	Finishing diets		
	Control	30-ByPro®	60-ByPro®
<i>Ingredient inclusion, % DM</i>			
SFC	63.72	63.72	63.72
WCGF	20.00	20.00	20.00
Alfalfa	4.00	4.00	4.00
CSH	4.00	4.00	4.00
Yellow Grease	3.00	3.00	3.00
Supplement ¹	2.00	2.00	2.00
Urea	0.68	0.68	0.68
Limestone	1.60	1.60	1.60
Premix ²	1.00	1.00	1.00
<i>Analyzed Composition³, % DM</i>			
DM, % as-is	78.70	78.40	78.10
CP	13.50	13.20	13.60
NDF	20.80	22.30	21.10
ADF	11.90	12.30	11.40
EE	5.10	5.40	5.20
Ash	5.73	5.69	5.64
Ca	0.65	0.74	0.69
P	0.38	0.43	0.43
K	0.74	0.85	0.82
S	0.17	0.19	0.18

¹Supplement contained (DM basis): NaCl, 15%; CuSO₄, 0.12%; MnSO₄, 0.08%; Se Premix 0.2%, 0.25%; SnSO₄, 0.21%; Vitamin A, 550000 IU/kg; Vitamin E, 875 IU/kg; Rumensin (30 g/ton of monensin, DM basis, Elanco Animal Health, Indianapolis, IN); Tylan (9 g/ton of tylosin, DM basis, Elanco Animal Health).

²Premix contained: cottonseed meal, for control diet; 0.33% tannic acid (ByPro®) for 30-ByPro® diet; and 0.67% tannic acid (ByPro®) for 60-Bypro® diet. Cottonseed meal was used as carrier.

³Analyzed composition from a commercial laboratory (Servi-Tech Laboratories, Amarillo, TX) with the exception of CP which was analyzed by Kjeldahl method in house.

Table 4. Effects of ByPro® (tannic acid) titration (g/steer/day) in steam-flaked corn-based diets on beef cattle growth performance.

Item	ByPro Inclusion			SEM ¹	Linear	Quadratic
	Control	30 g	60 g			
Initial BW, kg	349	349	349	7.51	0.38	0.33
FsBW, kg ²	598	599	601	6.09	0.72	0.90
Adj.FsBW, kg ³	597	601	599	5.91	0.87	0.53
DMI, kg/d						
d 0 to 35	8.99	9.24	9.14	0.08	0.14	0.05
d 0 to 70	9.57	9.82	9.82	0.15	0.2	0.47
d 0 to 105	9.71	9.99	10.09	0.18	0.07	0.63
d 0 to 140	9.82	9.99	10.02	0.15	0.38	0.73
d 0 to end	9.90	10.16	10.27	0.16	0.06	0.65
ADG, kg						
d 0 to 35	2.07	2.14	2.13	0.05	0.44	0.33
d 0 to 70	1.82	1.90	1.90	0.05	0.16	0.53
d 0 to 105	1.87	1.90	1.94	0.04	0.17	0.89
d 0 to 140	1.84	1.86	1.87	0.03	0.52	0.96
d 0 to end ⁵	1.62	1.64	1.65	0.04	0.51	0.98
Adj. d 0 to end ⁴	1.62	1.66	1.64	0.04	0.65	0.38
G:F						
d 0 to 35	0.23	0.232	0.233	0.005	0.75	0.99
d 0 to 70	0.19	0.193	0.194	0.004	0.45	0.81
d 0 to 105	0.193	0.19	0.192	0.003	0.84	0.50
d 0 to 140	0.187	0.186	0.187	0.003	0.87	0.76
d 0 to end	0.164	0.161	0.161	0.002	0.33	0.71
Adj. d 0 to end ⁴	0.163	0.163	0.159	0.002	0.17	0.51

¹ Standard error of the mean.

² Final shrunk body weight. 4% shrink was applied to final live body weight.

³ Carcass-adjusted FsBW calculated from hot carcass weight divided by the average dressing percent across treatments (62.14%) and adjusted by a 4% shrink.

⁴ Carcass-adjusted ADG and G:F from carcass-adjusted final shrunk BW, initial BW, and days on feed.

Table 5. Effects of ByPro® (tannic acid) titration (g/steer/day) in steam-flaked corn-based diets on beef cattle carcass characteristics.

Item	ByPro Inclusion			SEM ¹	Linear	Quadratic
	Control	30 g	60 g			
Hot carcass weight, kg	387	390	387	3.82	0.86	0.52
Dressing percent ²	62.08	62.42	61.91	0.28	0.66	0.19
Fat Thickness, cm	1.48	1.55	1.46	0.10	0.87	0.43
LM area, cm ²	93.42	92.70	94.60	2.20	0.57	0.58
Marbling score ³	48.42	47.75	48.67	1.18	0.87	0.55
KPH	2.01	2.1	2.05	0.03	0.38	0.12
Calculated Yield Grade	2.96	3.1	2.9	0.18	0.73	0.29
<i>Quality Grade⁴</i>						
Premium Choice, %	32.0	40.1	35.1	8.09	0.76	0.44
Choice, %	52.0	48.8	52.1	8.33	0.99	0.72
Upper Choice, %	85.5	90.3	88.6	5.58	0.62	0.55
Select, % ⁴	14.5	9.7	11.4	5.58	0.54	0.61
<i>Liver score</i>						
Total, %	17.03	8.26	8.46	5.87	0.85	0.13
A+, % ⁵	4.30	4.21	6.21	3.55	0.68	0.80
A, % ⁶	7.65	3.66	11.32	4.81	0.48	0.17

¹ Standard error of the mean.

² Dressing percent was calculated using non-shrunk final BW/HCW.

³ 30 = sight; 40 = small; 50 = modest; 60 = moderate; 70 = slightly abundant.

⁴ Quality grade, USDA personnel.

⁵ Only A+

⁶ Includes A+, A, A-. Does not include condemned or flukes.

Table 6. Effects of ByPro® (tannic acid) titration (g/steer/day) in steam-flaked corn-based finishing diets on beef cattle apparent total tract digestibility.

Item	ByPro Inclusion			SEM ¹	Linear	Quadratic
	Control	30 g	60 g			
Intake ¹ , kg/d						
DM	9.46	9.78	10.01	0.289	0.07	0.86
OM	8.83	9.16	9.33	0.269	0.08	0.73
Starch	4.42	4.62	4.57	0.132	0.29	0.27
CP	1.25	1.24	1.31	0.038	0.30	0.20
NDF	2.09	2.16	2.18	0.062	0.17	0.60
Fecal output, kg/d						
DM	2.46	2.36	2.65	0.158	0.25	0.17
OM	2.29	2.14	2.42	0.161	0.41	0.13
Starch	0.08	0.10	0.13	0.020	0.03	0.55
CP	0.38	0.37	0.44	0.026	0.04	0.09
NDF	1.28	1.20	1.24	0.112	0.71	0.54
Apparent total tract digestibility, %						
DM	73.91	75.74	73.42	1.58	0.75	0.14
OM	74.03	76.51	73.51	1.71	0.94	0.09
Starch	98.20	98.00	97.20	0.42	0.03	0.54
CP	69.90	70.13	66.38	2.02	0.09	0.26
NDF	38.45	44.25	42.97	4.87	0.36	0.41
Diet Composition, %, DM basis						
DM, % <i>as-is</i>	78.16	78.31	78.44			
OM	93.36	93.70	93.23			
Starch	46.70	47.30	45.60			
CP	13.40	12.70	13.05			
NDF	23.63	23.57	23.31			

¹Intake during the digestibility measurement period of the experiment (d 90 through 96 of the feeding period).

Table 7. Effects of ByPro® (tannic acid) titration (g/steer/day) on fecal nitrogen volatilization

Item	ByPro® Inclusion			SEM ¹	Linear	Quadratic
	Control	30 g	60 g			
Nitrogen volatilization, % Intake	55.17	55.42	51.6	3.74	0.91	0.39
N volatilization, g/steer/d	111	110	106	7.21	0.56	0.81
N volatilization Kg/steer	17.04	16.69	16.45	4.81	0.69	0.96

¹ Standard error of the mean.

Table 8. Instrument color values (L^* , a^* , b^* , hue angle and saturation), discoloration ratio, and relative pigment proportions (% oxymyoglobin, deoxymyoglobin, metmyoglobin) of overwrapped packaged *L. lumorum* steaks during retail display as influenced by inclusion level (0,30,60 g/steer/d) of ByPro® (tannic acid) into steam flaked corn based diets of finishing beef cattle.

Variable	Treatment			SEM	P-value
	Control	30-ByPro®	60-ByPro®		
<i>Instrument Color</i>					
L^*	42.37	39.84	39.67	3.50	0.33
a^*	27.22	26.43	26.51	2.70	0.41
b^*	23.60	23.06	23.06	2.21	0.16
Hue Angle ¹	41.36	41.73	41.36	0.65	0.48
Saturation ²	36.09	35.16	34.95	3.36	0.07
<i>Pigment Proportion³</i>					
Discoloration Ratio	6.66	9.50	6.55	4.46	0.60
Metmyoglobin	19.46 ^b	20.53 ^{ab}	23.29 ^a	4.88	<0.01
Deoxymyoglobin	17.27	16.55	16.08	2.37	0.17

¹ Hue angle = $\tan^{-1} b^*/a^*$

² Saturation index = $(a^{*2} + b^{*2})^{1/2}$

³ Relative pigment proportions (%; oxymyoglobin, metmyoglobin, deoxymyoglobin) were calculated using reflectance values obtained by a portable spectrophotometer (Hunter Miniscan XEPlus, model MSXP-4500C) and formulas outlined by the American Meat Science Association (2012).

Table 9. The effects of retail display day on the instrument color values (L^* , a^* , b^* , hue angle and saturation), discoloration ratio, and relative pigment proportions (% deoxymyoglobin, metmyoglobin) of overwrapped packaged *L. lumbrorum* steaks from cattle fed steam flaked corn based diets with ByPro® (tannic acid) incorporated at 0, 30, or 60 g/steer/d.

Variable	Retail Display Day								SEM	P-Value
	0	1	2	3	4	5	6	7		
<i>Instrument Color</i>										
L^*	41.47	43.06	41.14	38.71	39.60	39.68	45.72	35.64	2.99	0.18
a^*	36.62 ^a	30.50 ^b	29.22 ^{bc}	28.90 ^c	25.20 ^d	22.89 ^e	19.63 ^f	20.80 ^f	2.73	<0.0001
b^*	30.20 ^a	24.28 ^b	24.43 ^b	24.86 ^b	21.98 ^c	20.80 ^d	18.62 ^e	20.77 ^d	2.22	<0.0001
Hue Angle ¹	39.36 ^{de}	38.43 ^e	39.99 ^d	40.78 ^{cd}	41.55 ^c	42.88 ^b	43.54 ^b	45.35 ^a	0.71	<0.0001
Saturation ²	47.50 ^a	39.00 ^b	38.12 ^b	38.13 ^b	33.49 ^c	30.98 ^d	26.42 ^e	29.58 ^d	3.38	<0.0001
<i>Pigment Proportion³</i>										
Discoloration Ratio	29.06 ^a	6.50 ^b	6.06 ^b	6.87 ^b	4.60 ^b	3.80 ^b	2.87 ^b	0.80 ^b	6.44	<0.0001
Metmyoglobin	4.81 ^f	13.00 ^e	15.79 ^e	16.13 ^e	23.85 ^d	29.37 ^c	34.82 ^b	40.61 ^a	4.96	<0.0001
Deoxymyoglobin	34.00 ^a	20.87 ^b	20.00 ^b	17.18 ^c	13.81 ^d	10.59 ^e	8.54 ^f	7.77 ^f	2.41	<0.0001

^{a-f} Least square means, within a row, lacking a common superscript letter differ ($P < 0.05$).

¹ Hue angle = $\tan^{-1} b^*/a^*$

² Saturation index = $(a^{*2} + b^{*2})^{1/2}$

³ Relative pigment proportions (%; oxymyoglobin, metmyoglobin, deoxymyoglobin) were calculated using reflectance values obtained by a portable spectrophotometer (Hunter Miniscan XEPlus, model MSXP-4500C) and formulas outlined by the American Meat Science Association (2012).

Table 10. Relative proportion of oxymyoglobin (%) during retail display in overwrapped packaged *L. lumberum* steaks from cattle fed steam flaked corn based diets with ByPro® (tannic acid) incorporated at 0, 30, or 60 g/steer/d.

Treatment	Retail Display Day							
	0	1	2	3	4	5	6	7
Control	70.62 ^{a,z}	65.61 ^{b,z}	63.93 ^{bc,z}	67.26 ^{ab,z}	61.15 ^{c,z}	60.12 ^{c,yz}	57.06 ^{c,yz}	50.50 ^{d,z}
30-ByPro®	69.77 ^{a,z}	66.06 ^{ab,z}	65.16 ^{b,z}	65.34 ^{b,z}	62.19 ^{b,z}	57.67 ^{c,y}	54.07 ^{c,y}	44.77 ^{d,y}
60-ByPro®	72.03 ^{a,z}	66.71 ^{b,z}	63.54 ^{b,z}	66.55 ^{b,z}	63.67 ^{b,z}	62.35 ^{bc,z}	58.80 ^{c,z}	49.70 ^{d,yz}

^{a-f} Least square means, within a row, lacking a common superscript letter differ ($P < 0.05$).

^{y-z} Least square means, within a column, lacking a common superscript letter differ ($P < 0.05$).

¹ Treatment \times Retail display day, $P = 0.0017$, SEM = 3.40.

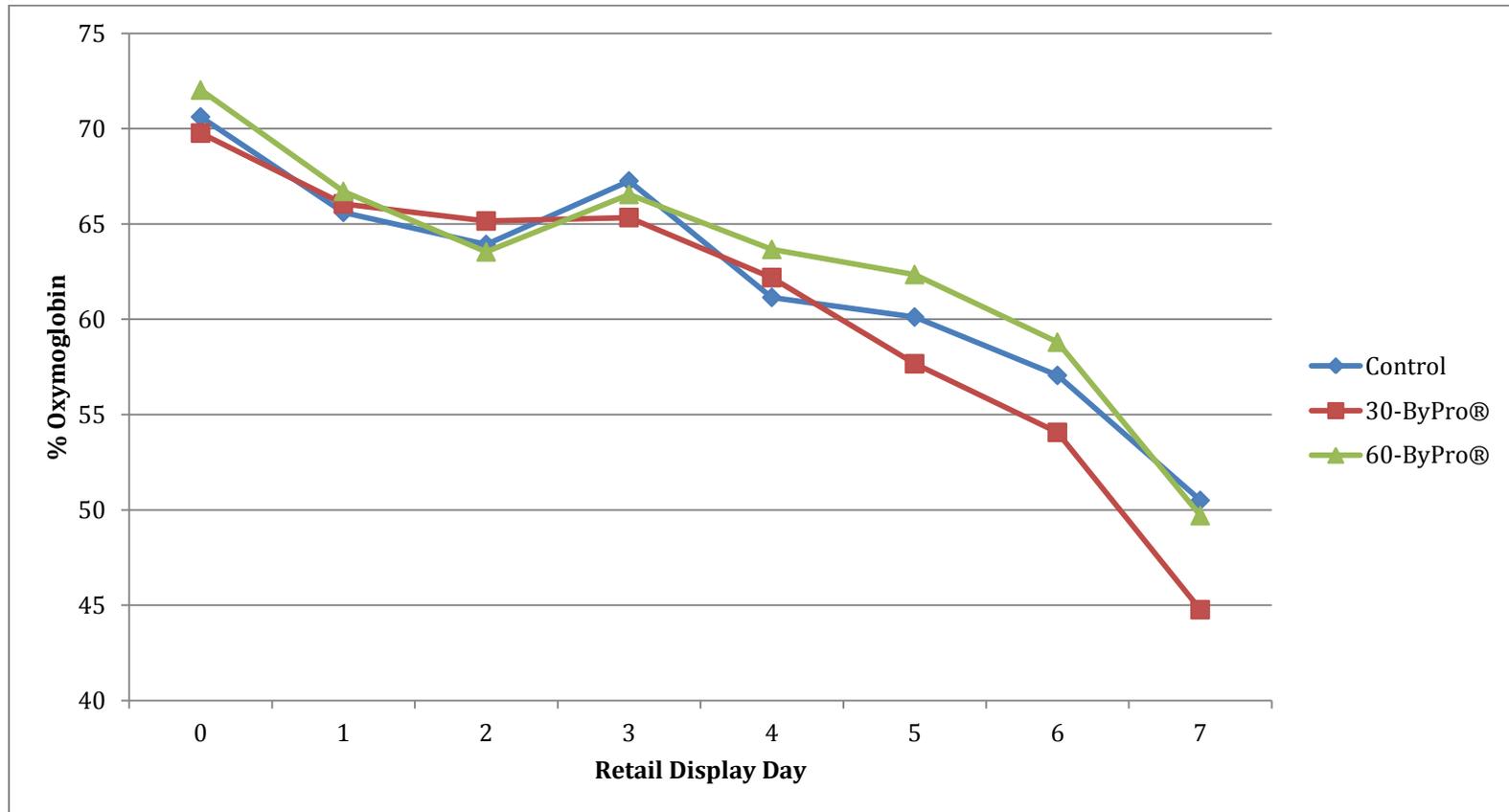


Figure 1. The effects of retail display day on the instrument color values (L^* , a^* , b^* , hue angle and saturation), discoloration ratio, and relative pigment proportions (% deoxymyoglobin, metmyoglobin) of overwrapped packaged *L. lumorum* steaks from cattle fed steam flaked corn based diets with ByPro® (tannic acid) incorporated at 0, 30, or 60 g/steer/d. ByPro® treatment and retail display length interacted ($P = 0.0017$, SEM = 3.40) to influence the proportion of oxymyoglobin present on the lean surface on day 5 through 7 where oxymyoglobin proportions from control and 6-ByPro® were greater than 30-ByPro®.

Table 11. Thiobarbituric acid reactive substances (TBARs; mg malondialdehyde/ kg meat) in 21 d aged beef strip loins from cattle fed steam flaked corn based diets with ByPro® (tannic acid) incorporated at 0, 30, or 60 g/steer/d.

Variable	Treatment			SEM	<i>P</i> -Value
	Control	30-ByPro®	60-ByPro®		
TBARs	0.3041	0.3186	0.3176	0.013	0.43
pH	5.427	5.393	5.380	0.124	0.77

CHAPTER V

CONCLUSIONS

The blend of tannic acid (ByPro®, Silva Team) evaluated in the current study does seem to influence greater feed intake in the beginning of the finishing phase when added to steam-flaked corn based finishing diets. Potential positive effect of ByPro® on growth performance may be further investigated for steers fed growing diets. Carcass characteristics were not negatively effected by ByPro® inclusion. For beef cattle on SFC-based finishing diets, the tannic acid blend provided by ByPro® only induced subtle (1 percentage unit) negative impact on apparent total tract starch digestibility, while OM digestibility tended to be improved due to possible positive effects on fiber digestibility. ByPro® fed to beef cattle on SFC-based finishing diets at 30 or 60/g/steer/d seems to not impact fecal N volatilization. It also did not seem that tannin blend inclusion provided by ByPro® had any influence on shelf life of overwrap packaged steaks. Higher inclusion levels of tannins in the diet may need to be included for them to have an influence on antioxidant activity and extend shelf life in meat.

LITERATURE CITED

- Arechiga, S.C., B.J. Cervantes, M.A. Espino, L.R. Flores, A. Camacho, J. A. Romo, and R. Barajas. 2011. Effect of length feeding additional tannins-extract on carcass traits of finishing-bulls. JAM. New Orleans, LA.
- American Meat Science Association. 2012. AMSA Meat Color Measurement Guidelines.
- AOAC. 1995. Official Method of Analysis. 16th ed. Association of Official Analytical Chemists, Arlington, VA.
- AOAC. 1997. Official Methods of Analysis. 16th ed. Association of Official Analytical Chemists, Arlington, VA.
- Barajas, R., B.J. Cervantes, S.C. Arechiga, M.A. Espino, L.R. Flores, A. Camacho, J.A. Romo. 2011a. Effect of length feeding additional tannins-extract on feedlot-performance of finishing-bulls. JAM. New Orleans, LA. (abstract)
- Barajas, R. B.J. Cervantes, A. Camacho, M. Verdugo, M.A. Espino, L.R. Flores, J.A. Romo, E.A. Velaquez, J.J. Lomeli. 2011b. Influence of addition of tannins-extract in low concentration of dietary dry matter on feedlot-performance of bulls. JAM. New Orleans, LA. (abstract).
- Barajas, R. B.J. Cervantes, M.A. Espino, A. Camacho, M. Verdugo, L.R. Flores, S.C. Arechiga, J.J. Lomeli, and J.A. Romo. 2012a. Influence of tannins extract addition on feedlot-performance of bulls fed sorghum-based diets. J. Anim. Sci. Vol. 90 (Suppl. 3):372-373 (abstract).

- Barajas, R. B.J. Cervantes, M.A. Espino, A. Camacho, M. Verdugo, L.R. Flores, J.J. Loneli, and J.A. Romo. 2012b. Effect of tannins extract supplementation on feedlot performance and plasma urea nitrogen of hearing bulls fed dry-ground corn-based diets containing corn-DDG and cane molasses. *J. Anim. Sci.* Vol 90 (Suppl. 3):600 (abstract)
- Bhat, T.K., B. Singh, O.P. Sharma. 1998. Microbial degradation of tannins—a current perspective. *Biodegradation*, 9:343–357
- Bi, J.L., G.W. Felton, J.B. Murphy, P.A. Howles, R.A. Dixon, C.J. Lamb. 1997. Do Plant Phenolics Confer Resistance to Specialist and Generalist Insect Herbivores? *J. Agric. Food Chem.* 45:5(11):4500-4504.
- Brooker, J.D., L.A. O’Donovan, I.K. Skene, K. Clark, L. Blackall, P. Muslera. 1994. *Streptococcus caprinus* sp. nov., a tannin-resistant ruminal bacterium from feral goats *Lett. Appl. Microbiol.*, 18:313–318
- Brunet, S., J. Aufrere, F. El Babili, I. Fouraste, H. Hoste. 2007. The kinetics of exsheathment of infective nematode larvae is disturbed in the presence of tannin-rich plant extract (sainfoin) both in vitro and in vivo. *Parasitology*. 134:1253-1262. Doi: 10.1017/S0031182007002533
- Camacho, A., B.J. Cervantes, M.A. Espino, M. Verdugo, L.R. Flores, J.A. Romo, R. Barajas. Influence of addition of tannins-extract in low concentration of dietary dry matter on carcass characteristics of bull-calves. *JAM.* New Orleans, LA. 2011. (abstract)

- Cannas, A. 2014. Tannins: fascinating but sometimes dangerous molecules. Dept of Animal Science- Plants Poisonous to Livestock. Cornell University College of Agriculture and Life Sciences.
- Cole, N.A., P.J. Defoor, M.L. Galyean, G.C. Duff, and J.F. Gleghorn. 2006. Effects of phase-feeding of crude protein on performance, carcass characteristics, serum urea nitrogen concentrations, and manure nitrogen of finishing beef steers. *J Anim Sci.* 84:3421-3432.
- Costabile, A., S. Sanghi, S. Martin-Pelaez, I. Mueller-harvey, G.R. Gibson, R.A. Rastall, A. Klinder. 2011. Inhibition of *Salmonella Typhimurium* by tannins in vitro. *Journal of Food, Agriculture & Environment.* 9.(1): 119-124.
- Domby, E.M., U.Y. Anele, K.K. Gautam, J.E. Hergenreder, A.R. Pepper-Yowell, M.L. Galyean. 2013. Interactive effects of bulk density of steam-flaked corn and concentration of Sweet Bran on feedlot cattle performance, carcass characteristics, and apparent total tract nutrient digestibility. *J. An Sci.* 92:1133-1143.
- Fernandez-Myakawa, M.E., A.M. Elizondo, E. Mercado. 2009. Tannins as a tool for the control of intestinal diseases produced by *Clostridium perfringens*. Instituto de Patobiologia, Instituto Nacional de Tecnologia Agropecuaria, Castelar. Buenos Aires, Argentina. Poster.

- Formanek, Z., J.P. Kerry, F.M. Higgins, D.J. Buckley, P.A. Morrissey, J. Farkas. 2001. Addition of synthetic and natural antioxidants to alpha-tocopheryl acetate supplemented beef patties: effects of antioxidants and packaging on lipid oxidation. *Meat Science*. 58:337-341. DOI: 10.1016/S0309-1740(00)00149-2
- Frutos, P., G. Hervás, F.J. Giraldez, and A.R. Mantecon. 2004. Review. Tannins and ruminant nutrition. *Spanish Jour of Agric Res*. 2(2), 191-202
- Garg S.K., Makkar H.P.S., Nagal K.B., Sharma S.K., Wadhwa D.R., and Singh B.. 1992. Oak (*Quercus incana*) leaf poisoning in cattle. *Vet. Hum. Toxicol.*34:161-164.
- Georgantelis, D., B. Georgios, P. Katikou, I. Ambrosiadis, D. J. Fletouris. 2007. Effect of rosemary extract, chitosan and alpha-tocopherol on lipid oxidation and colour stability during frozen storage of beef burgers. *Meat Science*. 75:2,256-264.
- Hagerman, A.E. 2002. *Condensed Tannin Structural Chemistry*. Miami University, Oxford, OH.
- Hagerman, A.E. 2010. *Hydrolyzable Tannin Structural Chemistry*. Miami University, Oxford, OH.
- Hartxfeld, P.W., R. Forkner, M.D. Hunter, A.E. Agerman. 2002. Determination of Hydrolyzable Taninns (Gallotannins and Ellagitannins) after Reaction with Potassium Iodate. *J. Agric. Food Chem*. 2002. 50:1785-1790.
- Hoste, H., F. Jackson, S. Athanasiadou, S.M. Thamsborg, S.O. Hoskin. 2006. The effects of tannin-rich plants on parasitic nematodes in ruminants. *Trends in Parasitology*. 22(6):253-261. DOI:10.1016/j.pt.2006.04.004

- Jerimiah, L. and L. Gibson. 2001. The influence on storage temperature and storage time on color stability, retail properties and case-life of retail-ready beef. *Food Res. Intl.* 34:815-826
- Jayanegara, A., E. Palupi. 2010. Condensed Tannin Effects on Nitrogen Digestion in Ruminants: A Meta-analysis from in Vitro and in Vivo Studies. *Media Peternakan.* 10:176-181.
- Krueger, W.K., H. Gutierrez-Banuelos, G.E. Carstens, B.R. Min, W.E. Pinchak, R.R. Gomez, R.C. Anderson, N.A. Krueger, T.D.A. Forbes. 2010. Effects of dietary tannin source on performance, feed efficiency, ruminal fermentation, and carcass and non-carcass traits in steers fed a high-grain diet. *AnimAnimal Feed Science and Technology* 159 (2010) 1-9.
- Krzywicki, K. 1979. Assessment of relative content of myoglobin, oxymyoglobin, and metmyoglobin at the surface of beef. *Meat Science* 3, 1-10.
- Langer, J.N. 2013. Antibacterial effects of condensed tannins on ruminant fecal bacteria. Master of Science Thesis. Tarleton State University. December.
- Li, Y.G., P. Larkin. 1996. The DMCA-HCl protocol and the threshold proanthocyanidin content for bloat safety in forage legumes. *J. Sci. Food Agric.*, 70:89-101.
- Lorenz, M.M., L. Alkhafadji, E. Stringano, S. Nilsson, I. Mueller-Harvey, P. Uden. 2013. Relationship between condensed tannin structures and their ability to precipitate feed proteins in the rumen. *J Sci Food Agric.* 94:963-968.

MacAdam, J.W., J. Brummer, A. Islam, G. Shewmaker. 2013. The Benefits of Tannin-Containing Forages. Utah State University Plants, soils, and climate.

AG/Forages/2013-03pr

Makkar, H.P.S., B. Singh, R.K. Dawra. 1988 Effect of tannin-rich leaves of oak (*Quercus incana*) on various microbial enzyme activities of the bovine rumen. Br. J. Nutr., 60:287–296

Mancini R.A., Hunt M.C. 2005. Current research in meat color. Meat Science. 71:100-121

Martinez, T.F., T.A. McAllister, Y. Wang, T. Reuter. (2006). Effects of tannic acid and quebracho tannins on in vitro ruminal fermentation of wheat and corn grain. J. Sci. Food Agric. 86:1244-1256.

McLeod, M.N. 1974. Plant tannins- Their role in forage quality. Nutr Abst Rev 44, 803-812.

Min, B.R., W.E. Pinchak, R. Merkel, S. Walker, G. Tomita, R.C. Anderson. 2008. Comparative antimicrobial activity of tannin extracts from perennial plants on mastitis pathogens. Sci. Res. Essays 3, 66-73.

Mueller-Harvey I., A.B. McAllan. 1992. Tannins. Their biochemistry and nutritional properties. In: Advances in plant cell biochemistry and biotechnology. Vol I (Morrieson I.M., ed). JAI Press Ltd., London (UK). P151-217.

Nerín, C., L. Tovar, D. Djenane, J. Camo, J. Salafranca, J.A. Beltrán, and P. Roncalés. 2006. Stabilization of beef meat by a new active packaging containing natural antioxidants. Jour of Agric and Food Chem 52:5598-5605

- Pennington, J.A.T., and R.A. Fisher. 2009. Classification of fruits and vegetables. *Jour of Food Comp and Ana* 22S:S23-S31.
- Porter, L.J. 1992. Structure and chemical properties of the condensed tannins. *Plant Polyphenols*. Plenum Press, New York. 245-246.
- Radhakrishnan, M.R. and J. Sivaprasad. 1980. Tannin Content of Sorghum Varieties and Their Role in Iron Bioavailability. *J.Agric. Food Chem.* 28:55-57.
- Reed, J.D. 1995 Nutritional Toxicology of Tannins and Related Polyphenols in Forage Legumes. *J Anim Sci.* 1995. 73:1516-1528.
- Selinger, L.B., C.W. Forsberg, K.J. Cheng. 1996. The rumen: a unique source of enzymes for enhancing livestock production. *Anaerobe*, 2:263–284.
- Santos-Buelga, C. and A. Scalbert. 2000. Proanthocyanidins and tannin-like compounds – nature, occurrence, dietary intake and effects on nutrition and health. *J. Sci. Food Agric.*, 80: 1094–1117. doi: 10.1002/(SICI)1097-0010(20000515)80:7<1094::AID-JSFA569>3.0.CO;2-1. Abstract
- Silanikove, N. 2000. The physiological basis of adaptation in goats to harsh environments. *Small Ruminant Res* 35, 181-193.
- Sindt, J.J., J.S. Drouillard, E.C. Titgemeyer, S.P. Montgomery, C.M. Coetzer, T.B. Farran, J.N. Pike, J.J. Higgins, R.T. Ethington. 2003. Wet corn gluten feed and alfalfa hay combinations in steam-flaked corn finishing cattle diets. *J Anim Sci.* 81:3121-3129.
- Singh, B., T.K. Bhat, O.P. Sharma. 2001. Biodegradation of tannic acid in an in vitro ruminal system. *Livestock Production Science* 68:259-262.

- Velasco, V. and P. Williams. 2011. Improving meat quality through natural antioxidants. Chilean J. of Agric. Research. 71(2) April-June.
- Velioglu, Y.S., G. Mazza, L. Gao, and B.D. Oomah. 1998. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. Journal of Agriculture and Food Chemistry 46:4113-4117.
- Zembayashi, M., Lunt, D.K., and Smith, S.B.. 1999. Dietary tea reduces the iron content of beef. Meat Sci.53:221-226.
- Zhu, J., L.J. Filippich, J. Ng. 1995. Rumen involvement in sheep tannic acid metabolism. Vet Hum Toxicol. 7(5)L436-440. (abstract)