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UofA
UNIVERSITY OF ARKANSAS
DIVISION OF AGRICULTURE

UNIVERSITY OF ARKANSAS
DALE BUMPERS COLLEGE
OF AGRICULTURAL, FOOD & LIFE SCIENCES

DISCOVERY

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Undergraduate Research Articles

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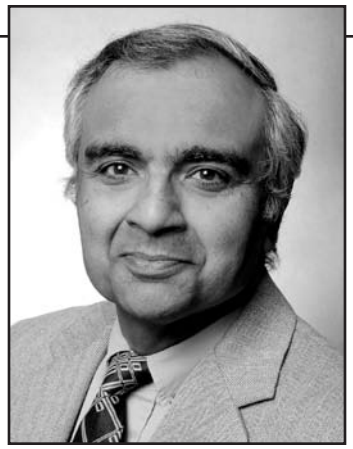
Cover: The fresh blackberry market in the U.S. has grown significantly in the past 10 years or so due in part to the development of new cultivars with improved firmness and longer shelf life. Worldwide, there has been a 45% increase in area planted by producers from 1995-2005. The paper in this issue by Carvalho et al. examines shipment and price trends from 1999-2008 and presents a history and characterization of this growing market. Cover design by Judy Howard. Photo by Fred Miller.

Letter from the Dean



Lalit Verma

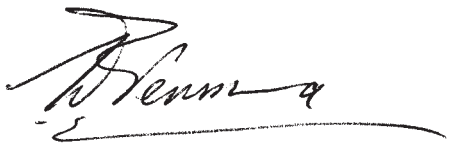
The Discovery undergraduate journal is one of the ways that Dale Bumpers College of Agricultural, Food and Life Sciences encourages students to engage in learning experiences beyond the classroom. The student authors are reporting on the results of research projects they have conducted with faculty mentors.

The Discovery journal provides a reporting outlet for our student scholars and scientists. It does not supersede publication elsewhere, but it does provide a forum for students and faculty to share their results and findings in a citable publication.

We encourage student research by awarding undergraduate research grants. Our students also have been very competitive for research and travel grants awarded by the University of Arkansas Honors College and the Arkansas Department of Higher Education. Many undergraduate research projects are designed to meet the requirements of an honors thesis in the Bumpers College Honors Program.

The nine articles in this 10th annual volume of *DISCOVERY* cover areas of crop management; animal science; agricultural marketing; dietetics; agricultural systems technology management; human nutrition; and food science.

We are proud to present these articles as examples of the research accomplishments of our undergraduate students. I heartily congratulate the student authors on their accomplishments and extend thanks to their faculty mentors.



Lalit R. Verma, Interim Dean and
Associate Vice President–Academic Programs

Processing and storage effects on the polyphenolic content and antioxidant capacity of conventional and sugar-free blueberry jams

Chelsey Castrodale[*]*, Luke Howard*[†]*, and Cindi Brownmiller*[§]

ABSTRACT

Fresh blueberries have received much attention due to their positive role in human health and disease prevention. The abundance of polyphenolics, namely anthocyanins and procyanidins, is thought to play an important role in health promotion. Due to seasonal availability and limited shelf-life, blueberries are commonly preserved and consumed in various thermally processed forms (jams, juices, canned whole fruit, and purées). Both conventional high sugar and sugar-free blueberry jams are available on the market, but no information is available on how different formulations, processing conditions, and storage of processed jams affect the retention of polyphenolics and antioxidant capacity found in fresh berries. In this study, fresh blueberries were processed into conventional and sugar-free jams, and stored for 6 months at 4°C and 25°C. Jams were analyzed 1 d after processing and after 2, 4, and 6 months of storage for anthocyanins and procyanidins by HPLC, percent polymeric color, and oxygen radical absorbing capacity (ORAC). Anthocyanins in conventional jams were more susceptible to degradation and polymerization during storage than anthocyanins in sugar-free jams, which may be associated with elevated sugar content. Higher levels of polymers in conventional jams resulted in higher ORAC values, indicating that the polymers formed during storage possess potent antioxidant capacity. However, more research is needed to characterize the anthocyanin polymers and assess their bioavailability. Anthocyanin pigments were much more stable in sugar-free jams indicating that the low calorie jams are a healthy alternative to conventional jams. Anthocyanins, procyanidins, and ORAC were better retained in jams stored at 4°C than at 25°C indicating blueberry jams should be stored under refrigeration in order to maximize retention of health-promoting antioxidants.

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[†]Luke Howard is the faculty mentor and a professor in the Department of Food Science.
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MEET THE STUDENT- AUTHOR

Chelsey Castrodale

I am from Little Rock and graduated from Mt. St. Mary Academy in 2005. I completed my freshman year of college at Miami University (of Ohio), and then transferred to the University of Arkansas. Since declaring my food technology major at the end of my sophomore year, I have loved every part of it. I have been awarded several departmental and university scholarships and honors, such as the Ozark Food Processors Association Scholarship, the Mid-South Institute of Food Technologists Scholarship, the Alumni Society Ring Scholar Award.

Upon my transfer, I founded the University of Arkansas Tennis Club and continued as club president through my senior year. The club has become very successful, with an active recreational team and a competitive team that travels the country playing other college club tennis teams. I am also active within Bumpers College and serve on the Student Advisory Board.

I plan to begin my M.S. degree program in food science here at the U of A in fall 2009 working under Dr. Andy Proctor, and I aspire to have a career product development with a major food corporation. I would like to thank Dr. Luke Howard, my honors mentor, and his lab team for all of their support these past few years.

INTRODUCTION

Antioxidants in foods have become a topic of great interest as a result of documented health-protective benefits. Blueberries have received much attention due to their high concentrations of polyphenolics including anthocyanins, procyanidins, and antioxidant activity. Berry anthocyanins and procyanidins are not only potent antioxidants, but they also contribute to the colors and astringency of the fruit. Antioxidants are beneficial to health due to their ability to scavenge free radicals and reduce oxidative stress. Oxidative stress is associated with many serious health conditions such as heart disease, cancer, Alzheimer's, and Parkinson's disease (Wu et al., 2004). Due to seasonal availability and limited shelf life, berries are commonly processed and consumed in various forms, including canned products, jams, jellies, juices, and syrups. Unfortunately, polyphenolic and antioxidant degradation is prevalent with processing of these products (García-Viguera et al., 1998; García-Viguera et al., 1999; Cabrita et al., 2000; García-Viguera and Zafrilla, 2001; Skrede and Wrolstad, 2002; Wicklund et al., 2004; Giusti and Jing, 2007).

There are many aspects of jam manufacturing that can have an adverse effect on berry polyphenolics and antioxidant capacity. Processing of raspberry jam resulted in anthocyanin losses of 21 to 39% in two cultivars of raspberries (García-Viguera and Zafrilla, 2001). Storage temperature of processed jams can also impact anthocyanin retention and antioxidant activity. Raspberry (García-Viguera et al., 1998) and strawberry (García-Viguera et al., 1999) jams stored at refrigerated temperature retained much higher levels of anthocyanins than jams stored at ambient temperature. Similarly, the antioxidant content of strawberry jam was more stable when stored at 4°C than at 20°C (Wicklund et al., 2004). The effect of sugar content on anthocyanin stability is controversial. High sugar levels were found to protect anthocyanins from degradation during storage of frozen strawberries (Wrolstad et al., 1990), which may be the result of reduced water activity (Skrede and Wrolstad, 2002). Conversely, studies on strawberry jams and strawberry preserves found that dehydration due to high sugar content initiated polymerization of anthocyanins with other polyphenolic compounds (García-Viguera et al., 1999; Abers and Wrolstad, 1979). The high temperature processing of jam inactivates enzymes involved in degradation of polyphenolics, therefore the only reactions taking place during storage are non-enzymatic. The primary non-enzymatic mechanism responsible for anthocyanin degradation during storage of jams involves a condensation reaction between anthocyanins and procyanidins that results in the formation of high molecular weight polymeric pigments. Unfortunately, it is not known whether the polymeric pigments are absorbed or have similar health benefits as the monomeric anthocyanins. However, if not completely inactivated by thermal treatments, polyphenol oxidase can react with anthocyanins to

produce polymeric compounds. This reaction decreases anthocyanin content and results in browning of pigments (Wesche-Ebeling and Montgomery, 1990; García-Viguera et al., 1999).

The objectives of this study were to determine the effects of jam processing and storage at ambient and refrigerated temperatures on the anthocyanin, procyanidin, and percent polymeric color of blueberry jam. The effects of sugar (conventional vs. sugar-free) on the degradation of polyphenolics during processing and storage were also determined. Antioxidant capacities of berry jams and other products have been examined, but in the only known study on blueberry jam (Kalt et al., 2000) the brand of jam, blueberry cultivar, and product formulation were unknown. Also, blueberries are only seasonally available, and it may be possible to obtain the health-promoting antioxidant properties through consumption of blueberry jam when fresh berries are unavailable. In addition, there is a lack of information on the effects of sugar on anthocyanin and antioxidant content of jams.

MATERIALS AND METHODS

Blueberry Jam Preparation and Processing. Conventional jams were prepared following the recipe on the Sure-Jell® Premium Fruit Pectin package for cooked blueberry jams. Sugar-free jams were prepared following the recipe on the Sure-Jell® Premium Fruit Pectin No Sugar Needed package for cooked no-sugar-added triple berry jams. Jars and screw bands were washed in a dishwasher before use. Lids were prepared by pouring boiling water over the lids and allowed to stand until use. Fresh blueberries were crushed using a stainless steel masher. For conventional blueberry jam, crushed fruit (1040 g) and 1 box of Sure-Jell® Premium Fruit Pectin (49 g) were added to a 6 qt (6.6 l) saucepot. The mixture was heated on high heat until it reached a full boil, then 900 g of sugar were added. The mixture was brought to a boil again and held for exactly 1 min. For sugar-free jams, crushed fruit (810 g) and 1 box of Sure-Jell® Premium Fruit Pectin No Sugar Needed (49 g) were added to a 6 quart (6.6 l) saucepot. The mixture was heated on high heat until it reached a full boil. After 1 min, 15 g of granulated Splenda were added. The jams were dispensed into 8-ounce glass jars, immediately capped, and pasteurized in boiling water for 10 min. Half of the conventional and sugar-free jams were stored at 25°C and the other half at 4°C.

Extraction of Anthocyanins. Jams were pureed using a common household blender (Black & Decker, Towson, Md.). Anthocyanins were extracted using the method of Hager et al. (2008) with modifications. Pureed jam (5 g) was homogenized with 20 mL of methanol/water/formic acid (60:37:3 v/v/v) using a Euro Turax T18 Tissuemizer (Tekmar-Dohrman Corp., Mason, Ohio). Fresh blueberries were pureed in a household blender (Black & Decker, Towson, Md.), and then 10 g of puree were homogenized using the same solvent. The samples were filtered through Miracloth (Calbiochem, La Jolla, Calif.), filter cakes were isolated, and the extraction repeated twice. The filtrates were pooled and adjusted to a final volume of 100 mL with extraction solvent.

HPLC Analysis of Anthocyanins. Anthocyanins were analyzed by HPLC using the method of Cho et al. (2004). Individual anthocyanin monoglycosides and acylated anthocyanin derivatives were quantified as delphinidin, cyanidin, petunidin, peonidin, and malvidin glucoside equivalents using external calibration curves of a mixture of anthocyanin glucosides obtained from Polyphenols Laboratories AS (Sandnes, Norway). Total anthocyanins were calculated as the sum of individual anthocyanin monoglycosides and acylated anthocyanin derivatives, with results expressed as mg per 100 g of original berry.

Extraction and Purification of Procyanidins. Jams were pureed using a common household blender (Black & Decker, Towson, Md.). Procyanidins were extracted using the method of Gu et al. (2002) with modifications. Pureed jams (5 g) were homogenized with 20 mL of acetone/water/acetic acid (70:29:0.5 v/v/v) using a Euro Turax T18 Tissuemizer (Tekmar-Dohrman Corp., Mason, Ohio). Fresh blueberries were pureed in a household blender (Black & Decker, Towson, Md.), and then 10 g of berry puree were homogenized using the same solvent. The samples were filtered through Miracloth (Calbiochem, La Jolla, Calif.), filter cakes were isolated and the extraction repeated twice. The filtrates were pooled and adjusted to a final volume of 100 mL with extraction solvent. Samples were purified by solid phase extraction using Sephadex LH-20. After equilibrating 3 grams of the Sephadex LH-20 with water overnight, the absorbent material was manually packed into 6 x 1.5 cm columns. Samples were prepared for purification by evaporating 25 mL of the crude extract under vacuum in a SpeedVac® (SPE 1010, Thermo Savant, Holbrook, N.Y.) at 25°C to approximately 7.5 mL. The concentrated extract was loaded onto the LH-20 column and the column was washed with 40 mL of 30% methanol in water to remove interfering compounds. Procyanidins were then eluted by washing the column with 80 mL of 70% acetone in water. The eluted procyanidins fraction was evaporated to dryness using a SpeedVac® and reconstituted with 2 mL of acetone/water/acetic acid (70:29.5:0.5 v/v/v). The reconstituted samples were filtered through 0.45 μm PTFE syringe filters prior to HPLC analysis.

HPLC Analysis of Procyanidins. Procyanidins were analyzed by HPLC using the method of Kelm et al. (2006). The procyanidin peaks were monitored by fluorescence detection with excitation at 276 nm and emission at 316 nm

using a Waters Model 474 fluorescence detector (Milford, Mass.). Individual procyanidins with degrees of polymerization from DP1 (monomer) through DP8 (octamer) were quantified using external calibration curves of cocoa procyanidin standard obtained from Mars Incorporated (Hackettstown, N.J.). Total procyanidins were calculated as the sum of individual procyanidins with results expressed as mg per kg of original berry.

Polymeric Color Analysis. Percent polymeric color was determined using the method described by Giusti and Wrolstad (2001). Sample extracts were diluted with deionized water to obtain an absorbance reading between 0.5 and 1.0 at 512 nm using an 8452A Diode Array Spectrophotometer (Hewlett Packard, Palo Alto, Calif.). For analysis, 0.2 mL of 0.90 M potassium metabisulfite was added to 2.8 mL diluted sample (bisulfite bleached sample) and 0.2 mL of deionized water was added to 2.8 mL diluted sample (non-bleached, control sample). After equilibrating for 15 min, samples were evaluated at = 700, 512, and 420 nm. Color density was calculated using the control sample according to the following formula:

Color density = $[(A_{420nm} - A_{700nm}) + (A_{512nm} - A_{700nm})] \times$ dilution Factor.

Polymeric color was determined using the bisulfite-bleached sample using the following formula:

Polymeric Color = $[(A_{420nm} - A_{700nm}) + (A_{512nm} - A_{700nm})]$.

Percent polymeric color was calculated using the formula:

% Polymeric Color = (polymeric color/color density) $\times$ 100.

Determination of Antioxidant Capacity. Antioxidant capacity was measured using the oxygen radical absorbing capacity (ORAC) assay of Prior et al. (2003). The assay was carried out using a FLUOstar microplate reader with fluorescein as fluorescent probe. Results were expressed as µmoles of trolox equivalents per g of original berry.*Calculations.* Anthocyanin, procyanidin, and ORAC levels in jams were converted to original berry weight using the following calculation:

$C_{product} * R = C_{berry}$,

where, $C_{product}$ = concentration of jam, R = ratio of mass of product produced to the mass of the original berry, and C_{berry} = concentration based on original berry weight. This conversion allowed for concentration and dilution effects to be accounted for and all products to be compared on an equivalent basis.

Sampling. Three jars each of conventional and sugar-free jams stored at 25°C and 4°C were sampled 1 d after processing, and after 2, 4, and 6 mo of storage.

Statistical Analysis. Effects of jam type, storage time, and storage temperature on total anthocyanins, total procyanidins, polymeric color, and ORAC were analyzed by analysis of variance (ANOVA) using JMP® software (SAS Inst. Inc., Cary, N.C.).

RESULTS AND DISCUSSION

Total Anthocyanins. Anthocyanin content of the jams was affected by storage time ($P<0.0001$), storage temperature ($P<0.0002$), and jam type ($P<0.0221$). Percent retention of anthocyanins compared to levels found in fresh blueberries was calculated to determine changes in total anthocyanin content of conventional and sugar-free blueberry jams (Fig. 1). One day after processing, 81% of the total anthocyanins in conventional blueberry jam were retained. This was consistent with a previous study on raspberry jam that reported anthocyanin retentions of 76 to 83% (García-Viguera et al., 1998). In sugar-free jam, 83% of total anthocyanins were retained after processing. After 2 mo of storage, there was a clear temperature effect on anthocyanin retention. Jams stored at 4°C retained 77% and 72% anthocyanins for conventional and sugar-free jams, respectively. Jams stored at 25°C had much lower retentions of 65% and 62% for conventional and sugar-free jams, respectively. After 4 mo of storage, an effect of sugar content on anthocyanin retention was evident, as sugar-free jam stored at 4°C had the highest retention of 64%. The remaining jams had similar retentions; conventional jam stored at 4°C and sugar-free jam stored at 25°C both retained 56% of anthocyanins, and 25°C conventional jam retained 55%. At the end of the 6 mo storage period, both temperature and sugar content had a significant effect on anthocyanin retention. Sugar-free jam stored at 4°C retained the greatest amount of anthocyanins (71%). The degradation of anthocyanins caused by the higher storage temperature of sugar-free jam seemed to be lessened by sugar content, because this jam had the same retention as conventional jam stored at 4°C (52%). Conventional jam stored at 25°C had the lowest anthocyanin retention (36%). Anthocyanins were found to be more stable when jams were stored at lower temperatures. These results were consistent with previous studies on strawberry and raspberry jam, where anthocyanins were found to be more stable when stored at lower temperatures (García-Viguera et al., 1998; García-Viguera et al., 1999). Additionally, it was found that sugar increased anthocyanin degradation, as sugar-free jams retained higher levels than conventional jams.

Total Procyanidins. Total procyanidin content of the jams was affected by storage time ($P<0.0001$) and storage temperature ($P<0.0001$), but not by jam type ($P=0.1614$). Procyanidin degradation was also determined by percent retention compared to fresh berries, and the trend over the 6 mo was similar to that of anthocyanins (Fig. 2), except sugar content had no effect on procyanidins. After initial

processing 80% of procyanidins were retained in conventional jam, and 89% were retained in sugar-free jam. Temperature effects were noticeable early with 4°C retentions of 62% for conventional jams and 64% for sugar-free jams after 2 mo. Jams stored at 25°C showed lower retentions of 53% for conventional and 51% for sugar-free jam. After 4 mo, procyanidin retention was significantly greater for jams stored at 4°C compared to those stored at 25°C. After 6 mo, procyanidin retention was affected by storage temperature. Sugar-free jam stored at 4°C had the highest retention (41%), and conventional jam stored at 4°C had the next highest retention (28%). These values were higher than the 25°C jam retentions of 16% and 20% for conventional and sugar-free jams, respectively. As with anthocyanins, higher storage temperature was detrimental to procyanidins.

Percent Polymeric Color. Percent polymeric color was measured to determine the amount of polymerized anthocyanins resistant to bleaching with sodium metabisulfite (Fig. 3). Higher percent polymeric color indicated the possible formation of anthocyanin-procyanidin polymers. Polymeric color was affected by storage time, storage temperature, and jam type ($P<0.0001$). One day after processing, all of the jams had approximately 7% polymeric color. After 4 mo of storage, a difference in storage temperatures was observed. Jams stored at 4°C had polymeric color values of 9% and 10% for conventional and sugar-free jams, respectively; but jams stored at 25°C had higher values of 17% and 13% for conventional and sugar-free jams, respectively. After 6 mo, it was evident that the presence of sugar resulted in a significant increase in anthocyanin polymerization though storage temperature was also causative. The 25°C conventional jam had the highest percent polymeric color of 42%. Conventional jam stored at 4°C had a polymeric color value of 26%, which was much lower than that of the 25°C jam, but still higher than the values of sugar-free jams. These data suggest that jams stored at 25°C had lower anthocyanin retentions as a result of the formation of anthocyanin-procyanidin polymers as indicated by percent polymeric color. Sugar also seemed to promote polymerization, as conventional jams had higher percent polymeric color values than sugar-free jams. Additionally, it is possible that Maillard Browning reaction products could have been formed in the presence of sugar and contributed to increased polymeric color values.

Antioxidant Capacity. Antioxidant capacity was measured by Oxygen Radical Absorbance Capacity (ORAC), and percent retentions were calculated to determine changes in antioxidant capacity (Fig. 4). ORAC was affected by storage time ($P<0.0114$), storage temperature ($P<0.0001$), and jam type ($P<0.0011$). After initial processing, retentions were minimally affected, with 90% and 79% retentions in conventional and sugar-free jams, respectively. The conventional jam stored at 4°C had the highest retention of antioxidant capacity over the 6 mo storage time, retaining 82% after 2 mo, and 85% after 6 mo. Sugar-free jam stored at 4°C also had somewhat high retentions after 2 mo (74%), and after 6 mo (80%). After 2 mo of storage, 25°C jams retained 70% and 61% of antioxidant capacity for conventional and sugar-free jams, respectively. These retentions rose slightly after 6 mo to 75% for conventional and 65% for sugar-free jams. Overall, conventional jams retained more antioxidant capacity than sugar-free jams, but 4°C jams retained more than 25°C jams. Even though conventional sugar jams showed higher anthocyanin degradation and percent polymeric color, they also had higher antioxidant capacities after 6 mo. It is possible that the antioxidant capacity lost from anthocyanin degradation was compensated by anthocyanin polymers formed, which still display antioxidant properties. However, these polymers are most likely large molecular weight compounds, and it is not known if they can be absorbed into the body to provide antioxidant benefits. This finding is in agreement with a study by Brownmiller et al. (2008), which found that antioxidant capacity of blueberry juices was stable during storage, during which time significant losses in total anthocyanins occurred, but polymeric color increased. Additionally, some Maillard browning compounds that could have formed may have antioxidant properties (Yilmaz and Toledo, 2005).

SUMMARY

Anthocyanins in conventional jams were more susceptible to polymerization during storage than anthocyanins in sugar-free, which may be associated with elevated sugar content. Higher levels of polymers in conventional jams resulted in higher ORAC values, indicating that the polymers formed during storage possess potent antioxidant capacity. However, more research is needed to characterize the anthocyanin polymers and assess their bioavailability. Anthocyanin pigments were much more stable in sugar-free jams indicating that the low-calorie jams are a healthy alternative to conventional jams. Blueberry jams should be stored under refrigeration in order to better retain polyphenolics and antioxidant capacity.

ACKNOWLEDGMENTS

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LITERATURE CITED

Abers, J.E. and R.E. Wrolstad. 1979. Causative factors of color deterioration in strawberry preserves during processing and storage. J. Food Sci. 44:75-81.

Brownmiller, C., L.R. Howard and R.L. Prior. 2008. Processing and storage effects on monomeric anthocyanins, percent polymeric color, and antioxidant capacity of processed blueberry products. J. Food Sci. 73:72-79.

Cabrita, L., T. Fossen, and Ø.M. Anderson. 2000. Colour and stability of the six common anthocyanidin 3-glucosides in aqueous solutions. Food Chem. 68:101-107.

Cho, M.J., L.R. Howard, R.L. Prior, R.L., and J.R. Clark, 2004. Flavonoid glycosides and antioxidant capacity of various blackberry, blueberry and red grape genotypes determined by high-performance liquid chromatography/mass spectrometry. J. Sci. Food Agric. 84:1771-1782.

García-Viguera, C. and P. Zafrilla. 2001. Changes in anthocyanins during food processing: influence on color. In Chemistry and Physiology of Selected Food Colorants. J.M. Ames and T. Hofmann, (eds). Oxford University Press, New York, N.Y., p. 56.

García-Viguera, C., P. Zafrilla, F. Artés, F. Romero, P. Abellán, and F.A. Tomás-Barberán. 1998. Colour and anthocyanin stability of red raspberry jam. J. Sci. Food Agric. 78:565-573.

García-Viguera, C., P. Zafrilla, F. Artés, F. Romero, P. Abellán, and F.A. Tomás-Barberán. 1999. Colour stability of strawberry jam as affected by cultivar and storage temperature. J. Food Sci. 64:243-247.

Giusti, M.M. and P. Jing. 2007. Natural pigments of berries: functionality and application. In Berry Fruit: Value-Added Products for Health Promotion. Y. Zhao (ed). CRC Press: Boca Raton, FL, pp. 104-166.

Giusti, M.M. and R.E. Wrolstad. 2001. Characterization and measurement of anthocyanins by UV-visible spectroscopy. In Current Protocols in Food Analytical Chemistry 1st ed., R.E. Wrolstad, T.T. Acree, and H. An. (eds). John Wiley & Sons, Hoboken, N.J., pp F1.2.1-F1.2.9.

Gu, L., M. Kelm, J.F. Hammerstone, G. Beecher, D. Cunningham, S. Vannozzi and R.L. Prior. 2002. Frac-tionation of polymeric procyanidins from lowbush blueberry and quantification of procyanidins in selected

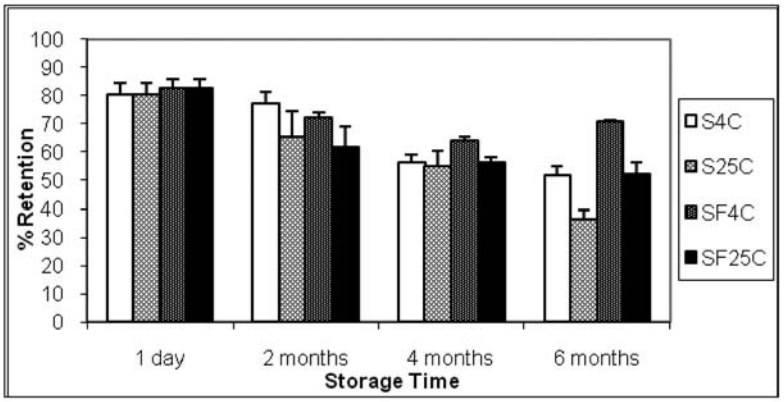

Fig. 1. Percent retention of total monomeric anthocyanins of blueberry jams at various storage times and temperatures. Bars represent standard error of the mean (n=3). S = conventional sugar jams, SF = sugar free jams, 4C = 4°C, and 25C = 25°C.

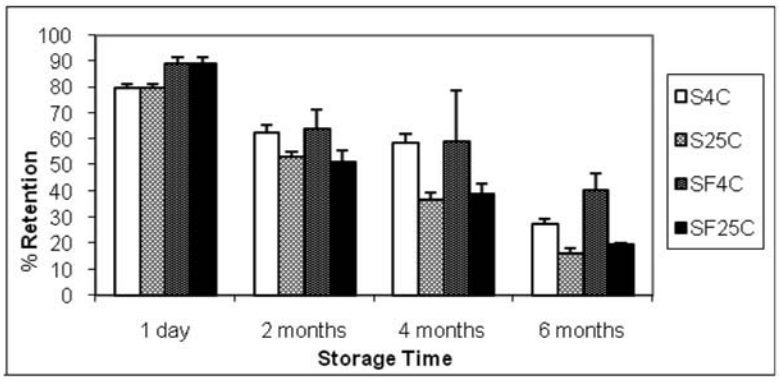

Fig. 2. Percent retention of total procyanidins of blueberry jams at various storage times and temperatures. Bars represent standard error of the mean (n=3). S = conventional sugar jams, SF = sugar free jams, 4C = 4°C, and 25C = 25°C

foods with an optimized normal-phase HPLC-MS fluorescent detection method. J. Agric. Food Chem. 50:4852-4860.

Hager, T.J., L.R. Howard and R.L.Prior. 2008. Processing and storage effects on monomeric anthocyanins, percent polymeric color, and antioxidant capacity of processed blackberry products. J. Agric. Food Chem. 56:689-695.

Jackman, R.L. and J.L.Smith. 1996. Anthocyanins and betalains. In Natural Food Colorants, 2nd ed., G.A.F. Hendry, and J.D. Houghton (eds). Blackie Academic & Professional: Glasgow, UK, pp. 244-309.

Kalt, W., J.E. McDonald and H. Donner. 2000. Antho-cyanins, phenolics and antioxidant capacity of processed lowbush blueberry products. J. Food Sci. 65:390-393.

Prior, R.L., H. Hoang, L. Gu, X. Wu, M. Bacchiocca, L. Howard, M. Hampsch-Woodill, D. Huang, D.B. Ou and R. Jacob. 2003. Assays for hydrophilic and lipophilic antioxidant capacity [oxygen radical absorbance capacity ($ORAC_{FL}$)] of plasma and other biological and food samples. J. Agric. Food Chem. 51:3273-3279.

Skrede, G. and R.E. Wrolstad. Flavonoids from berries and grapes. 2002. In Funtional Food: Biochemical and Processing Aspects, J. Shi, G. Mazza and M.L. Maguer (eds). CRC Press: Boca Raton, FL, pp. 71-133.

Wesche-Ebeling, P. and M.W. Montgomery. 1990. Strawberry polyphenoloxidase: its role in anthocyanin degradation. J. Food Sci. 55:731-734, 745.

Wicklund, T., H.J. Rosenfeld, B.K. Martinsen, M.W. Sundfør, P. Lea, T. Bruun, R. Blomhoff and K. Haffner. 2004. Antioxidant capacity and colour of strawberry jam as influenced by cultivar and storage conditions. LTW 38:387-391.

Wrolstad, R.E., G. Skrede, P. Lea and G. Enersen, G. 1990. Influence of sugar on anthocyanin pigment stability in frozen strawberries. J. Food Sci. 55:1064-1065, 1072.

Wu, X., G.R. Beecher, G.M. Holden, D.B. Haytowitz, S.E. Gebhardt and R.L. Prior. 2004. Lipophilic and hydrophilic antioxidant capacities of common food in the United States. J. Agric. Food Chem. 52:4026-4037.

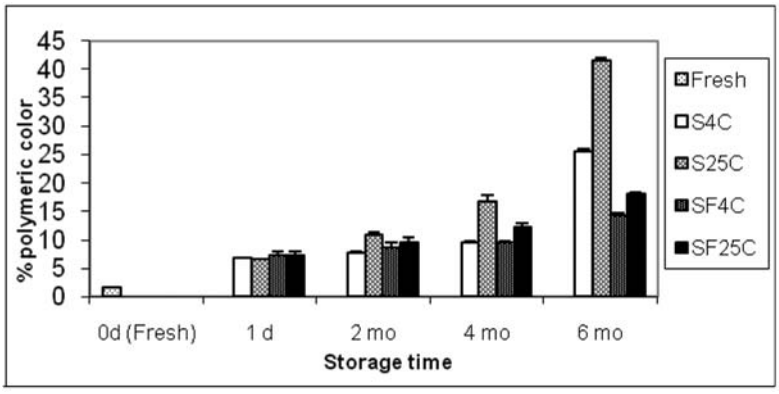

Fig. 3. Percent polymeric color of fresh blueberries and blueberry jams at various storage times and temperatures. Bars represent standard error of the mean (n=3). S = conventional sugar jams, SF = sugar free jams, 4C = 4°C, and 25C = 25°C.

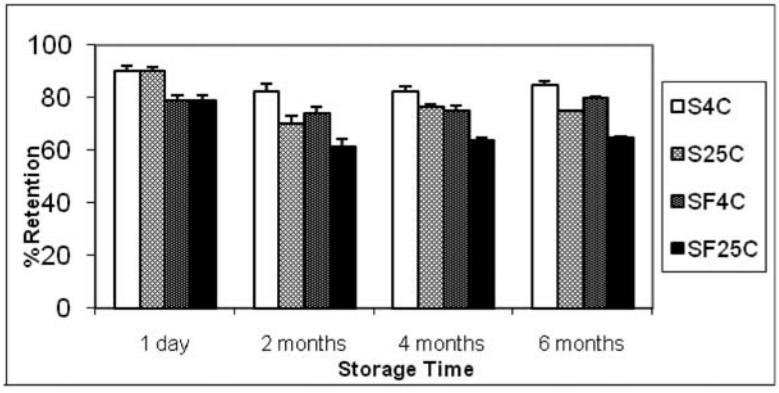

Fig. 4. Antioxidant capacity (ORAC) of blueberry jams at various storage times and temperatures. Bars represent standard error of the mean (n=3). S = conventional sugar jams, SF = sugar free jams, 4C = 4°C, and 25C = 25°C

History of development and characterization of the U.S. blackberry market

Thais F. Carvalho[*], *John R. Clark*[†], *and Michael R. Thomsen*[§]

ABSTRACT

The fresh blackberry market within the United States has expanded significantly in the past 10 years based on the development of new cultivars with improved firmness and longer shelf life, permitting their shipment over long distances. Currently, blackberries maintain a nearly continuous presence on the shelves of grocery stores across the U.S., which was uncommon a decade ago. Increased consumption of blackberries is due to increased consumer desire for improved nutrition and diet along with expanded availability. Worldwide, producers have increased production with a 45% increase in area planted from 1995 to 2005. Further expansion has occurred since then. This examination of the market history and its characterization was intended to highlight major aspects of the blackberry market as it changed from 1999 to 2008. The primary source of information was from shipments and price data maintained by the USDA's Agricultural Marketing Service. With these data, it was possible to track trends in the U.S. fresh blackberry market. Specifically, growth in blackberry shipments from major domestic and international production regions and price trends in major U.S. terminal markets were characterized. Results show fresh blackberry shipments increased by 530% from 2000 to 2008. The largest volume of blackberry shipments originated from California and Mexico. Blackberry prices at all terminal markets had a similar seasonal pattern. In October and November, prices were highest as these months represent the end of California's production season and the very beginning of Mexico's. The lowest prices occurred in May, when there was a very high level of Mexican imports, and often in July when domestic production was greatest.

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MEET THE STUDENT- AUTHOR

Thais Carvalho

I am an international student from Brazil. I am currently a senior at Sao Paulo State University in Botucatu and am pursuing a B.S. degree in Agronomy.

I first came to the University of Arkansas as an exchange student in Fall 2008. I was impressed by the University and decided to do my internship here, a requirement from my college in Brazil in order to graduate. I was accepted as an intern by Drs. John Clark and Michael Thomsen, who proposed my blackberry marketing history research project. This has been a great experience for me, and I received many benefits from my time at the University of Arkansas. It has been a pleasure to work with the support and guidance of both of these great faculty members. I plan to continue with graduate studies after I receive my bachelor's degree.

INTRODUCTION

The fresh blackberry market in the United States has changed significantly in the past 10 years. In the early 1990s, the market was concentrated in pick-your-own and pre-picked blackberries, sold at the farm or at farmers' markets (Clark, 2005). Blackberries were rarely seen in retail stores; one of the principal reasons was that fruits did not have adequate shelf life and firmness to be shipped across long distances. Due to breeding improvements, new cultivars have been released with improved quality, which make cross-continental shipment possible. Today blackberries originate from both domestic and foreign producers and are shipped to major terminal markets within the U.S., where they are purchased for sale at retail outlets.

Their presence in retail markets has met a growing consumer demand for fresh blackberries, which is due to fruit characteristics that reflect the changing trends in consumer behavior. Consumer concern for nutritional properties and health benefits has increased and many purchasers have begun opting for more healthy food. According to Halvoren *et al.* (2006), blackberries had the highest antioxidant content per serving size among all the food categories evaluated in their study[1], with 5.57 mmol/serving. They also have anti-inflammatory, anti-neurodegenerative, and anti-cancer properties (Seeram *et al.*, 2006). These health benefits have been used by the blackberry industry to promote consumption (U.S. Fed News Service, 2007). In addition to these benefits, blackberry cultivars in the U.S. market are now sweeter (Clark, 2005) and more appetizing, further expanding consumer acceptance.

The objectives of the research were to characterize the fresh blackberry market and pricing in the U.S. and describe changes from 1999 to 2008 using data available from the Agricultural Marketing Service of the United States Department of Agriculture (USDA-AMS).

MATERIAL AND METHODS

Data were gathered from USDA-AMS and compiled using SAS 9.2 (SAS, Cary, NC) to obtain aggregate measures of shipment volume of domestic and international fresh blackberries to U.S. markets. Also, price behavior at terminal markets was characterized using Microsoft Excel® 2007 software (Microsoft Corporation, Redmond, WA) for charts and tables. In addition, seasonality of blackberry prices was characterized using Hannan's (1963) harmonic model. This analysis permitted an estimation of prices at terminal markets. The USDA-AMS price data used in the study consist of a time series of weekly

[1]The Halvoren et al. (2006) study included fruits and fruit juices, vegetables and vegetable products, spices and herbs, nuts and seeds, chocolates and sweets, ready-to-eat cereals, desserts and cakes, berries and berry products, beverages, fast foods, soups, sauces, dressings, infant foods and beverages, legumes, snacks, grain and grain products, dairy products, mixed food entrees, fats and oils, meats and meat products, poultry and poultry products, fish and seafood, and eggs and egg dishes.

price observations over 10 years. With 52 weekly observations per year, there were 520 potential price observations available for analysis in each terminal market.[2] Following Hannan (1963), the subsequent model is estimated: Eq. (1)

$$P_t = \propto_0 + \propto_1 t + \sum_{k=1}^{26} \beta_k \cos_{tk} + \sum_{k=1}^{26} \gamma_k \sin_{tk} + \in_t$$

where $\propto_0$ is an intercept term, $\propto_1$ is the trend term, β_k and γ_k are seasonal coefficients and $\in_t$ is an error term. Regressors in the model consist of *t*, the trend variable; and cos_{tk} and sin_{tk}, variables that are used to characterize seasonality over the 52 weeks of the year and are computed as $cos\left(\frac{2\pi k}{52}\right)$ and $sin\left(\frac{2\pi k}{52}\right)$, respectively. The model in equation 1 was estimated by ordinary least squares using SAS's REG procedure.

RESULTS AND DISCUSSION

Fresh Blackberry Market Shipments

From 2000, the initial year that USDA-AMS had complete data for blackberries, to 2008, total fresh blackberry shipments increased 530% (Table 1). Fresh blackberry shipments from abroad and within the U.S. by origin are shown in Fig. 1, illustrating large growth of fresh blackberries in the marketplace over the past 10 years. Imports from Mexico were responsible for much of this growth, increasing more than nine times during this period (Table 1 and Fig.1). Mexican fruit was available at retail stores from October until July, coinciding with much of American producers' "off-season", which occurs during autumn, winter and early spring months (Clark, 2005). Mexican berries serve a role of great importance because they keep blackberries available in grocery stores the entire year, enabling consumer demand to be met year-round.

The large increase in the amount of berries sourced from Mexico represents a significant development in the blackberry market. In the mid and late 1990s, major international shipments to the U.S. were from Chile and Guatemala (Clark, 2005). However, Guatemala's production area decreased 63% from 1995 to 2005 (Strik *et al.*, 2007), which resulted in a lower quantity of blackberries shipped to the U.S. during the first years of the study period (Table 1). Costs of transporting Chilean blackberries to the American market are high, and Chilean berries have been supplanted by Mexican berries as a result (Clark, 2005).

Historically, Costa Rica has shipped blackberries to the U.S., but the quantity has typically been small because most of their production is processed and consumed locally (Strik *et al.*, 2007). As shown in Table 1, Costa Rican shipments steadily declined over the past nine years, and in 2008, no berries from this country were recorded in the USDA-AMS data.

According to Clark (2005), many domestic producers began to target their product to fresh markets due to the success of Mexican berries during the "off-season." Domestic blackberries are mostly provided by producers in California, Oregon, Texas, Arkansas, Georgia, North Carolina, Washington, Virginia, and Ohio.

Oregon had the highest overall blackberry production among the states. However, around 90% of that total production is processed (Strik *et al.*, 2007). Although the amount of fresh blackberries actually shipped from Oregon is low, there was an increase in berry shipments from 2000 to 2008 of over 200% (Table 1). Interestingly, Oregon producers shifted the beginning of their market window back one month from early July in 2000 to early August in 2008. This shift was a good strategy and enabled Oregon producers to command higher prices by reaching the market when California's blackberries had a decreased market presence.

After Mexico, California was the second-largest source of fresh-market blackberry shipments in the U.S. California berries were available in the retail market from May to early October. Though still far behind the quantity from Mexico, shipments from California increased more than 200% during the study period (Table 1).

In the mid 2000s, growers in states east of the Rocky Mountains, such as Arkansas, Georgia, and North Carolina, invested in blackberries for the fresh market and increased their production areas. From 1995 to 2005, Arkansas had a 60% increase and Georgia 300% (Strik *et al.*, 2007). Production from these states was largely designated for fresh markets, but quantity shipped from these states is not available in USDA-AMS data because almost all producers contract with a broker/shipper directly and do not report volumes to USDA-AMS (J.R. Clark, University of Arkansas, and John Shelford, Shelford Consulting, personal communication).

Fresh Blackberry Prices at Terminal Markets

The terminal (wholesale) market is a physical location in a metropolitan area where a product is sold by wholesalers to retailers or other large users in wholesale lots (AMS–USDA). Terminal market prices do not reflect producers' actual prices because they include costs of transportation, commissions paid to shippers/brokers, and other costs incurred between the farm and terminal market (John Shelford, Shelford Consulting, personal communication). However, terminal market prices do reliably show price performance over the study period and seasonal behavior of prices over the year. As an illustration, blackberry

[2]Occasionally prices were not reported and so some markets had missing values for price in one or more weeks.

prices from the Boston terminal market are shown in Fig. 2. Prices in other U.S. terminal markets exhibited similar patterns (data not shown).

The relatively stable long-term trend in prices is notable given the tremendous increase in fresh blackberry shipments and imports into the U.S. market. The normal effect of a supply increase of this magnitude would be a decrease in prices. That prices have not weakened is likely the result of aggressive marketing, good fruit quality, and increased consumer acceptance and consumption of blackberries. Table 2 reports selected results from Hannan's (1963) harmonic model for five terminal markets. The trend coefficients in Table 2 provide statistical evidence that price levels remained strong despite large growth in production. In Boston and Dallas, the positive and statistically significant trend coefficient shows that prices actually strengthened over the study period. It should be noted that in both of these markets the trend coefficient is small and shows only a five to ten cent increase in the price per pound over the entire study period. Chicago is the only market showing a negative trend over the study period. However, this is not statistically different from zero and is again very small in magnitude.

Another interesting feature of blackberry prices is their seasonality. Due to space limitations, numerous coefficients for the seasonal sine and cosine terms of Hannan's (1963) model are not reported. However, Table 2 does show that the seasonal model explains a significant portion of the variance in prices in each market. values ranged from 33% to 46% and the F statistics from each model are highly significant. In other words, seasonality explains from 1/3 to nearly 1/2 of the variation in blackberry prices observed in terminal markets over the study period. The seasonal pattern predicted by the harmonic model is shown for the Boston market in Fig. 3. The heavy, solid line shows the seasonal pattern predicted from Hannan's (1963) model and the thinner, dashed lines show actual price values during the most recent three years. Prices were lowest during January, May, and December and coincide with a high level of Mexican imports during this portion of the year. Lower prices also occurred in early August, the month that coincided with greatest domestic production. Highest prices were observed in October. October represented the end of California's production season and the beginning of Mexico's, when shipments of blackberries to fresh markets were at their lowest levels.

Despite relatively favorable long-term price trends in the presence of tremendous growth in production, grower profitability is susceptible to supply changes that occur within a given market window. A good case in point occurred in 2008 and affected domestic blackberry growers. As shown in Fig. 3, prices were much lower than would have been predicted during the June to August window. This was likely due to a very high level of fresh blackberry production and availability in the market that occurred in 2008 during the domestic season. Referring back to Fig. 1, evidence of this can be seen from the large increase in shipments from California in 2008 compared to 2007 and earlier years. As shown in Fig. 3, prices went down in late June and then reversed course in late August when the large supply of domestic fruit was moved out of the marketplace. This price anomaly was certainly of concern to blackberry growers and demonstrates the important role of seasonal supply patterns.

CONCLUSIONS

This study showed that fresh retail marketing of blackberries expanded greatly throughout the past 10 years with a significant increase of domestic production and importation, which demonstrated a shift in the countries of origin in the late 1990s. An interesting observation was that, even with expanded production, prices did not weaken substantially. This suggested a corresponding increase in consumer demand, which was likely due to better quality and flavor achieved by new cultivars and growing consumer awareness of health benefits associated with blackberries.

Mexican blackberries have an important function in the U.S. market and Mexican production keeps blackberries available in grocery stores throughout the entire year, which in turn helps to augment consumers' responsiveness and enables blackberries to become a routine purchase item.

Prices differed from terminal market to terminal market and over the production season, depending on shipment volumes and origin. Generally, prices were higher in September and October, months that corresponded to the lowest market supply levels; this coincides with the end of California's production and the beginning of Mexico's. Prices are lower in May, July, and November when supply from major production origins are the largest.

By shedding light on market characteristics, especially showing when and how blackberries from each origin are marketed and the resulting price dynamics, it is hoped that this study will enable producers to better develop strategies to address their production opportunities in late summer to early fall to take full advantage of higher price potential. Some breeding programs are already working to develop new varieties to address this market opportunity. The University of Arkansas Fruit Breeding Program has developed fall-fruiting (primocane) blackberries, which could expand production and supply in this high-priced season. This new blackberry produces fruit on new-growth canes for a harvest period that occurs during the fall, advantageously altering the production season.

Further research to collect blackberry production and marketing data from states east of the Rocky Mountains, such as Arkansas, Georgia, and North Carolina, would be interesting to demonstrate these states' real importance and position in the U.S. blackberry market.

ACKNOWLEDGMENTS

I would like to thank Dr. John Clark for this research opportunity along with financial support, help, advice, and patience during this project. I would also like to express gratitude to Dr. Michael Thomsen for extensive help, advice, and instruction in so many different areas of agricultural economics. It was a pleasure to work with both of these faculty members. I am also grateful to my friend Barrett Boone for assistance and editing, particularly for language, of my writing.

LITERATURE CITED

Clark, J.R. 2005. Changing times for eastern United States blackberries. HortTechnology 15:491-494.

Clark, J.R. 2009. University of Arkansas, personal communication.

Halvorsen, B.L., M.H. Carlsen, K.M. Phillips, S.K. Bohn, K. Holte, D.R. Jacobs, and R. Blomhoff. 2006. Content of redox-active compounds (ie, antioxidants) in foods consumed in the United States. Am. J. Clin. Nutr. 84:95–135.

Hannan, E.J. 1963. The estimation of seasonal variation in economic time series. J. Am. Statistical Assoc. 58:31- 44.

Seeram, N.P., L.S. Adams, Y. Zhang, R. Lee, D. Sand, H.S. Scheuller, and D. Heber. 2006. Blackberry, black raspberry, blueberry, cranberry, red raspberry, and strawberry extracts inhibit growth and stimulate apoptosis

Table 1. Fresh blackberry shipment to U.S. markets from 2000 to 2008 (in million pounds) (USDA-AMS).

	Mexico	California	Guatemala	Oregon	Chile	Costa Rica	Total
2000	5.90	3.32	2.06	0.90	-	0.46	12.64
2001	7.81	3.29	2.05	1.04	-	0.71	14.90
2002	9.02	3.59	1.81	1.41	0.31	0.31	16.45
2003	13.07	1.96	1.43	0.86	0.10	0.27	17.69
2004	16.99	4.22	2.20	0.95	0.13	0.13	24.62
2005	22.69	3.29	2.98	1.54	0.05	0.16	30.71
2006	32.61	4.58	2.89	1.85	0.14	0.07	42.14
2007	43.49	5.56	5.09	1.70	0.09	0.04	55.97
2008	53.37	7.23	4.33	1.85	0.07	-	66.85

Table 2. Selected estimates from Hannan's (1963) harmonic seasonal price model by terminal market.[1]

Statistic	Atlanta	Boston	Chicago	Dallas	Los Angeles
Number of observations	516	520	497	519	520
R^2	0.331	0.337	0.330	0.429	0.466
F	4.31	4.44	4.11	6.60	7.66
degrees of freedom	462	466	443	465	466
Intercept	5.42	4.94	4.77	4.29	3.913
t-ratio	59.52	44.77	47.51	44.21	42.87
Trend coefficient	0.0003	0.0013	-0.0002	0.0023	0.0003
t-ratio	1.13	3.41	-0.49	6.98	1.03

[1]Seasonal coefficients and corresponding t-ratios are not reported.

of human cancer cells in vitro. J. Agric. Food. Chem. 54: 9329-9339.
Shelford, J. 2009. Shelford Consulting, personal communication.
Strik, B.C., J.R. Clark, C.E. Finn, and M.P. Banados. 2007. Worldwide blackberry production. HortTechnology 17:205-213.
United States Department of Agriculture, Agricultural Marketing Service (USDA-AMS). 2008. Fruit & Vegetable Market News. <http://www.marketnews.usda.gov/portal/fv> Accessed Dec. 20, 2008.
U.S. Fed News Service. 2007. Oregon remains nation's leader in blackberry production. <http://proquest.umi.com/pqdweb?did=1306676341&Fmt=3&clientId=13929&RQT=309&VName=PQD> Accessed Jan. 05, 2009.

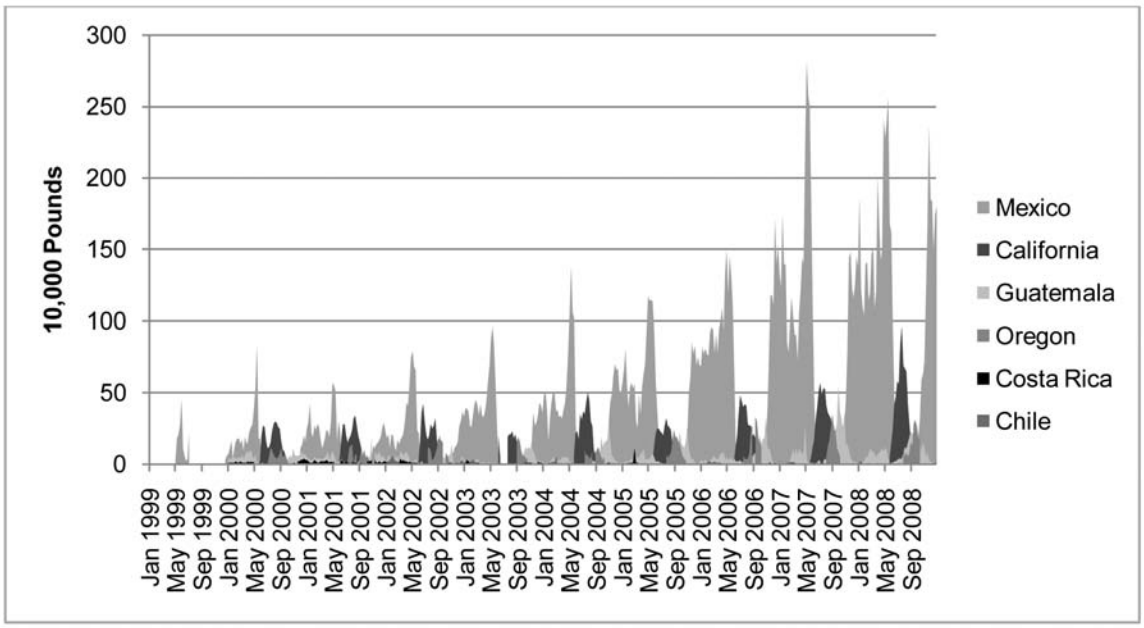

Fig. 1. Blackberry shipments to U.S. markets from 1999 to 2008 by origin (USDA-AMS).

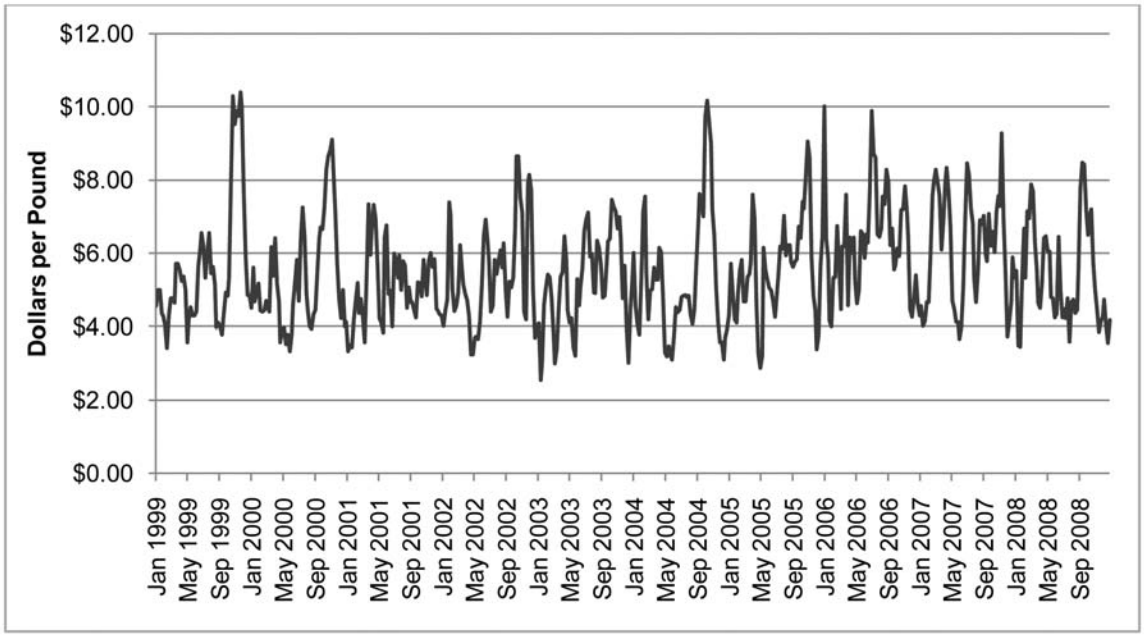

Fig. 2. Weekly fresh blackberry prices ($/lb) in the Boston terminal market (1999-2008) (USDA-AMS).

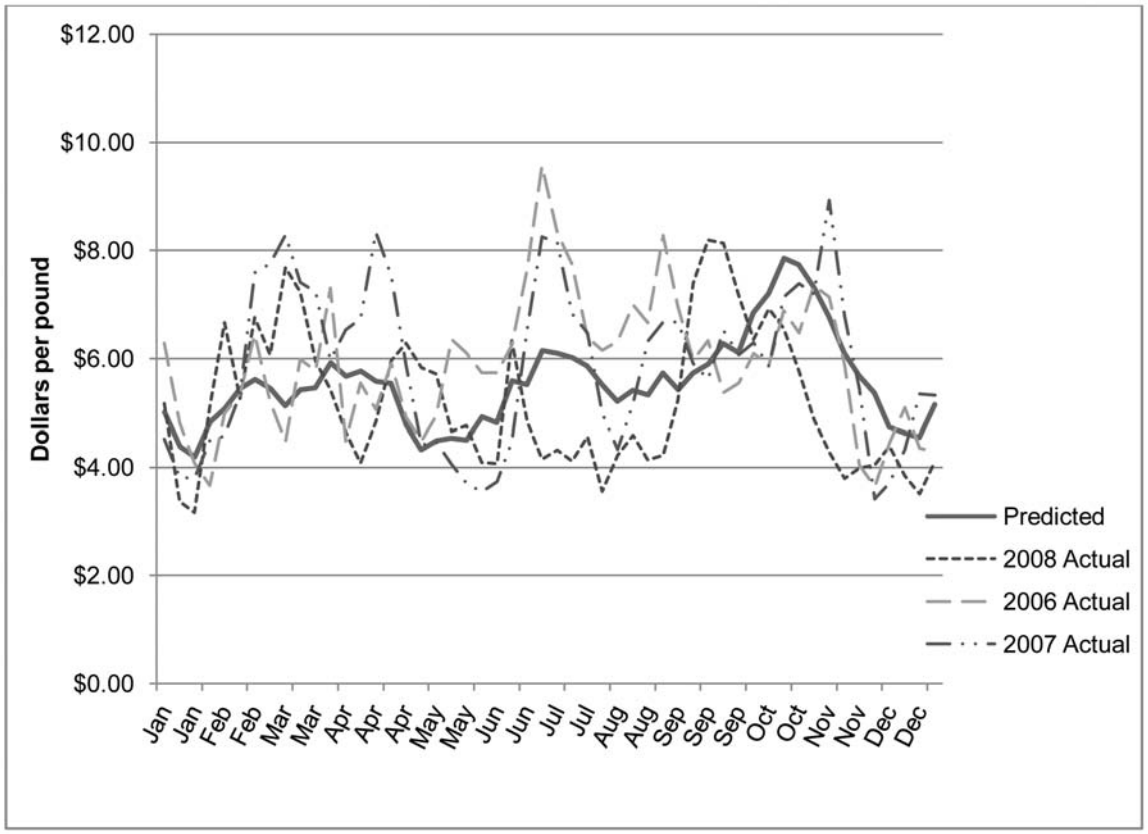

Fig. 3. Estimate of fresh blackberry prices in the Boston terminal market using the harmonic seasonal model (USDA-AMS).

Effectiveness of zinc given intra-nasally or orally to newly received stocker cattle against bovine respiratory disease and effects on growth performance

Amy R. Guernsey[*], *Beth Kegley*[†], *Jeremy G. Powell*[§], *Doug L. Galloway*[‡], *Alicia C. White*[**], *and Steve W. Breeding*[††]

ABSTRACT

Beef calves (n = 88) were purchased from regional auction barns and delivered as a single group. Upon arrival, cattle were assigned to eight pens. Pens were assigned randomly to one of three treatments; two pens received 3 mL of a nasal spray solution (10.8 mg Zn/mL) into each nostril using a single-use nasal atomizer; three pens received 40 mL of an oral drench (16.25 mg Zn/mL), and three pens received no Zn at processing (negative control). Appropriate treatments were administered at processing on d 0 of the 43-d study. After treatment, cattle were worked and housed so they did not have fenceline contact with any other pens. Cattle were observed daily and rectal temperatures were taken to monitor morbidity. Nasal membranes of four randomly selected calves/pen were swabbed prior to any treatment on d 0 and then on d 1, 2, 4, and 7. Those treated with intra-nasal Zn at processing had lower average daily gain for the first 28 d as compared to controls (P = 0.02) or oral Zn (P = 0.07). Final body weight did not differ. Treatments had no effect on percentage of morbid calves. Treatments had an effect on bacterial cultures from swabs; fewer (P ≤ 0.04) *Escherichia coli*, -*Streptococcus* spp., and *Staphylococcus* spp. colonies were cultured from cattle receiving the intra-nasal Zn. Bacterial cultures indicated reduced numbers of microbes in the nasal passages after treatment with intra-nasal Zn, but Zn treatments did not benefit overall morbidity or growth rates of stressed cattle.

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[†]Beth Kegley is a professor in the Department of Animal Science and is the mentor for the project.
[§]Jeremy Powell is an associate professor in the Department of Animal Science.
[‡]Doug Galloway is a program associate in the Department of Animal Science.
[**]Alicia White is a program associate at the Animal Diagnostic Laboratory.
[††]Steve Breeding is a veterinarian at the Animal Diagnostic Laboratory.

MEET THE STUDENT- AUTHOR

Amy Guernsey

I grew up in Joplin, Mo., and graduated from Joplin High School in 2005. Soon after, I ventured south to pursue an animal science degree at the University of Arkansas. I have made the most of my time as a Razorback, enjoying football games, exploring nearby Devil's Den State Park and staying active in the Pre-Veterinary Club and Block and Bridle. Additionally, I have worked in the animal science nutrition laboratory, a local veterinary clinic, and completed an internship in the Edinburgh Zoo (Scotland).

With the tremendous help of Dr. Beth Kegley, Dr. Jeremy Powell, and Doug Galloway, I finished an undergraduate honors research project funded by the Dale Bumpers College of Agricultural, Food, and Life Sciences and the University of Arkansas Honors College. After earning my bachelor's degree I will be attending veterinary school with the hopes of becoming either a mixed or zoo animal medical practitioner. I would like to thank all my family and friends for their ongoing support.

INTRODUCTION

Zinc, an essential dietary trace mineral, has been shown to be required for proper cell function and overall health of an organism. Although Zn may be found in bone or soft tissue, there is no homeostatic mechanism to mobilize this supply. Because of this, a steady intake of this mineral must be available in an individual's diet (Vruwink et al., 1993; Wintergerst et al., 2006). Beef cattle raised on forages are often deficient in Zn, so it is usually supplemented to them (Greene, 2000). Zinc is involved with DNA expression and consequently, protein synthesis and enzyme action. Zinc forms DNA binding proteins known as "fingers" (Klug, 2005), these independently folding domains are found on proteins and help bind the protein to control regions of a gene during the passage of an RNA polymerase molecule (Castro and Sevall, 1993). It is estimated that there are 2,000 transcription factors that need Zn for such structural integrity (Prasad, 2007). Zinc's role as a cofactor in enzymes involved in DNA synthesis and transcription is applicable to the expression of genes in many cell types, including those involved in immune response (Castro and Sevall, 1993). In fact, Zn is important for the expression of gene IL-2 in HUT-78 cells. This in turn contributes to expansion and maintenance of thymocyte and peripheral T cell populations, generation of antiviral and antitumor-specific cytotoxic T cells, delayed type hypersensitivity responses, and upregulation of Natural Killer lyric activity (Prasad, 2007). Also notable is Zn's role in protective enzymes such as antioxidants. For example, it is an integral part of superoxide dismutase, which acts as a 'scavenger' for free radicals (Hughes, 2000).

Another aspect of Zn's role in immunology is that this trace mineral actually has some antiviral properties as well. There is evidence to suggest that "adequate intakes of vitamin C and Zn ameliorate symptoms and shorten duration of respiratory tract infections including the common cold" (Wintergerst et al., 2006). A cold is caused by one of 200 types of rhinoviruses. An infection begins when one of these enters the nasal mucosa of a human or animal, from which it is "transported by mucociliary action to the nasopharynx" and proceeds as a more widespread infection (Cohen, 2006). For a rhinovirus to enter the nasal epithelium, it must bind to a cellular receptor, intracellular adhesion molecule-1 (ICAM-1). Zinc acts as "a competitive inhibitor of ICAM -1 in both rhinovirus particles and nasal epithelium" which essentially disrupts the virus's ability to penetrate the cell and replicate (Cohen, 2006). Additionally, because Zn inhibits the binding of leukocyte function associated antigen to ICAM-1 receptor sites, there is a reduction in inflammatory responses associated with colds (Cohen, 2006).

Recognizing Zn's potential, drug companies have developed throat lozenges and intranasal sprays, which aim to reduce the severity and duration of a cold by applying the Zn ion directly to the site of rhinovirus infection (Cohen, 2006). Numerous studies have been conducted on the effectiveness of these products. For lozenges, the best re-

sults were obtained when taken "immediately upon experiencing symptoms" and "taken around the clock (Cohen, 2006)." Similarly, nasal sprays seemed most effective when administered within 24 h of onset of symptoms (Cohen, 2006). These studies determined that the overall effectiveness of throat lozenges and nasal sprays is dependent upon the concentration, rather than the total amount of zinc ions as it is applied directly to mucosa (Wintergerst et al., 2006). This gives it the most contact with ICAM-1 receptors (Cohen, 2006). However taken as a whole, most of these studies were inconclusive at best (Wintergerst et al., 2006).

Bovine respiratory diseases cost farmers in the form of medication, time, quality and quantity of end product (losses due to death or decreased performance) (Bagley, 1997). In its upper-respiratory form, bovine respiratory disease is similar to the common cold in humans with symptoms such as coughing, fever, eye discharge, decreased appetite, and difficulty breathing (Bagley, 1997). It can be caused by a combination of stress and viral or bacterial infection (Bagley, 1997). In the case of a viral infection, no effective treatment can be offered; antibiotics are used only to combat secondary infections. A producer's best option in controlling this disease is to vaccinate against some of the viruses that initiate the syndrome (Richey, 1994). Alternative routes of vaccination such as intra-rectal and intra-nasal products aim to "generate protective antibody responses at mucosal surfaces" (Sedgmen et al., 2004). Very little research has been conducted on the use of products delivered to the mucosal surface in large animals (Sedgmen et al., 2004). The objectives of our research were to determine whether mucosal applications of Zn solutions could positively affect health and average daily gain of cattle susceptible to bovine respiratory disease, and to explore the effectiveness of intra-nasal and drench Zn applications in combating viral and bacterial loads.

MATERIALS AND METHODS

For this 43 d study, 88 male beef calves averaging 228 kg initial BW were obtained from regional sale barns. Upon receiving (d 0 of the study), cattle were processed as normal. They were assigned a unique ear identification tag and branded with the supplier's initial. Cattle were vaccinated for respiratory viruses including infectious bovine rhinotracheitis (IBR), bovine respiratory syncytial virus (BRSV), bovine viral diarrhea (BVD), and parainfluenza$_3$ (PI_3) (Cattle Master Gold FP5, Pfizer Animal Health, New York, N.Y.) and clostridial diseases (Covexin 8, Shering Plough Animal, Omaha, Neb.). An antihelmenthic was administered for internal parasites (Cydectin, Fort Dodge Animal Health, Fort Dodge, Iowa), and external parasites were also addressed (Double Barrel VP ear tags, Schering-Plough Animal Health, Summit, N.J.). Cattle were tested for persistent infection-with BVD (PI-BVD) by taking ear notch samples and shipping the samples to CattleStats in Oklahoma City, Okla., for analysis. Bulls were castrated using Callicrate bands (No-Bull Enterprises, St. Francis, Kan.). All cattle were sorted by sex and assigned randomly to 8 pens. Pens were assigned randomly to 1 of 3 treatments. These treatments were administered on d 0: Twenty two cattle (2 pens) received 3 mL of a nasal spray solution (10.8 mg Zn as Zn acetate/mL of 0.9% saline solution) into each nostril using a single-use nasal atomizer; 33 cattle (3 pens) received 40 mL of an oral drench (16.25 Zn as Zn acetate/mL of 0.9% saline solution), and 33 cattle (3 pens) received no Zn at processing to serve as a negative control.

Cattle were housed on eight 0.42-ha grass paddocks and were given ad libitum access to bermudagrass hay. They were offered a daily grain supplement of 1.8 kg as fed/d. This supplement consisted of 68% corn, 28% dried distillers grain, and vitamin and mineral premixes. The diet met and/or exceeded all nutritional requirements for protein and minerals (including Zn) as set by the NRC 1996.

To monitor morbidity, cattle were observed daily. Those that were coughing, appeared lethargic, or had ocular or nasal discharge were pulled from the group to take their rectal temperatures. If the temperatures exceeded 40°C, calves were considered morbid and a pre-planned regimen of antibiotics was administered. An initial treatment of florfenicol (Nuflor, Schering-Plough Animal Health, Summit, N.J.) was given first. Morbid calves were checked again 48 h later. If the re-check temperature was 40°C or higher, a second treatment of enrofloxacin (Baytril, Bayer HealthCare LLC, Animal Health Division, Shawnee Mission, Kan.) was given. After another 48 h, the rectal temperature was checked again. If it was still at or above 40°C, the last antibiotic of ceftiofur crystalline-free acid (Excenel, Pfizer Animal Health, New York, N.Y.) was administered daily for 3 d. No further antibiotics were offered after this final treatment. The rectal temperatures of all cattle were also taken on d 0, 1, 2, 3, 4, and 7 to monitor average trends.

Performance was monitored by observing body weight gain and supplement intake. Cattle were weighed on d 0, 1, 2, 3, 4, 7, 14, 28, 42, and 43 before supplement was offered. Any refusals of the grain supplement were weighed back daily.

To monitor viral and bacterial loads, the nasal membranes of 4 calves in each pen were swabbed prior to any treatment on d 0 and then on d 1, 2, 4, and 7. Viral swabs were packed on ice and immediately shipped via overnight courier to the Oklahoma State University Center for Veterinary Health Sciences (Stillwater, Okla.). Bacterial swabs were taken directly to the University of Arkansas Division of Agriculture Veterinary Diagnostic Laboratory (Fayette-

ville, Ark.) and cultured 24 h on five different media. Each swab was plated on a blood agar of 5% sheep blood, a Columbia CNA agar of 5% sheep blood, a chocolate agar, MacConkey agar, and a hektoen enteric agar. Laboratory personnel monitored and gave qualitative scores to these plates the following day.

Performance and morbidity data were analyzed using the mixed procedure of SAS (SAS Institute, Inc., Cary, N.C.). The model included treatment, gender (arrived as steer or bull), whether or not the calf's nasal membranes were swabbed, and all interactions. Degrees of freedom were calculated using the Kenward-Roger procedure. The random statement included pen, and for repeated measures, the model also included day and its interactions. Bacterial scores were analyzed using the GENMOD procedure of SAS. The model included treatment, gender, day, and all interactions. Binomial distribution of data and Type 3 analysis were specified. The means were generated with the frequency procedure.

RESULTS AND DISCUSSION

There were no differences in supplement intake ($P = 0.97$) or final body weights ($P = 0.15$). However, rates of gain varied between treatment groups (Table 1). Cattle that received the Zn nasal spray had lower average daily gain for the first 28 d of the study when compared to the control and oral Zn treatment groups ($P = 0.04$). Average daily gain up to 42 d reflected similar results. The Zn nasal spray treatment group again had lower gain when compared to the control group ($P = 0.06$), but the oral Zn treatment group was intermediate.

Although we randomly assigned cattle to treatment groups, those receiving the Zn nasal spray happened to have higher initial rectal temperatures (Fig. 1) (treatment by day interaction, $P = 0.01$). There were no other differences in rectal temperature observed. Likewise, there were no differences in percentage morbidity, number of calves pulled, or medication costs (Table 1). There was a 73% morbidity rate, but this was not different due to Zn treatments ($P = 0.43$). One calf on the control treatment died during the study.

We found numerous species of bacteria (Table 2), four of which are notable. *Pasteurella multocida* was by far the most prevalent in the cultures, and its occurrence seemed to be affected by a treatment by day interaction ($P = 0.07$; Fig. 2). There were treatment differences for three other species of bacteria (Fig. 4). Cattle that received Zn nasal spray had fewer ($P \leq 0.04$) colonies of *Escherichia coli*, -*Streptococcus* spp., and *Staphylococcus* spp.

There are no virus results to report. Although we packed and shipped our swabs exactly as instructed by Oklahoma State University, there were no viruses detected on any of them by the time they arrived.

In exploring why we obtained these results, it has been suggested that the cattle receiving the Zn nasal spray solution were under more stress than those in the other treatment groups. The nasal spray apparatus was awkward for the handler to use and for the animal to receive. The extra time spent handling the heads of the cattle may have increased stress which in turn could have suppressed the immune system, negatively impacting performance. As mentioned earlier, however, there were no recorded differences in morbidity between treatment groups. Additionally, cattle from each group had members that experienced the similar stressor of having their nasal membranes swabbed. When comparing cattle that were swabbed to those that were not, there were no differences detected in morbidity or growth performance.

In humans, anosmia, or a loss of sense of smell, has been noted as a potential side effect of using Zn nasal sprays (Cohen, 2006). If this were to occur in the cattle, decreased appetites may have also resulted. We observed no differences among treatment groups for grain supplement intake. However, we had no way of measuring hay consumption. There may have been differences in total feed intake that went undetected.

Finally, it appears that the Zn nasal spray had some antimicrobial effects. The question remains as to whether or not this was a positive outcome. Two of the more notable species found, *Pasteurella multocida* and *Escherichia coli*, are gram-negative bacteria. As such, they release endotoxins upon their death, potentially causing inflammation in the host animal. Additionally, by altering the natural flora of the mucosal membranes, the cattle may have become more susceptible to infection by more detrimental microbes. Killing the normally non-pathogenic bacteria of the nasal passages may have done more harm than good. While these were not the results we expected, they are interesting nonetheless. It does seem that these particular Zn applications had no positive impact on growth performance or against bovine respiratory disease in stressed cattle.

In conclusion, bacterial cultures indicated a reduced number of microbes in the nasal passages of cattle that received Zn nasal spray. However, neither Zn application appeared to have a positive impact on average daily gain or bovine respiratory disease in stressed cattle.

ACKNOWLEDGMENTS

Financial support for this project was provided by grants from the University of Arkansas Honors College and the Dale Bumpers College of Agricultural, Food, and Life Sciences Undergraduate Research Program. Also the assistance of Pete Hornsby, Carlee Jamison, John Richeson, and Jim Coffey is greatly appreciated.

LITERATURE CITED

Bagley, C.V. 1997. Bovine Respiratory Disease. Utah State University Extension Animal Health Fact Sheet. Electronic Publishing July 1997.

Castro, C.E. and J.S. Sevall. 1993. Zinc deficiency, chromatin structure, and gene expression. In: S. Cunningham-Rundles (ed.) Nutrient Modulation of the Immune Response. Marcel Dekker: New York, N.Y.

Cohen, D.A. 2006. The efficacy of zinc lozenges and zinc nasal sprays in the treatment of the common cold. Topics in Clinical Nutrition 21:355-361.

Falchuk, K.H. 1993. Zinc in developmental biology: the role of metal dependent transcription regulation. In: A.S. Prasad (ed). Essential and Toxic Trace Elements in Human Health and Disease: An Update. Wiley-Liss, Inc.: New York, N.Y.

Greene, L.W. 2000. Designing mineral supplementation of forage programs for beef cattle. J. Anim. Sci. 77:1-9.

Hughes, D.A. 2000. Dietary antioxidants and human immune function. Nutrition Bulletin 25:35-41.

Klug, A., S. Iuchi and N. Kuldell. 2005. The Discovery of zinc fingers and their practical applications in gene regulation: A personal account. In: S. Iuchi and N. Kuldell (ed). Zinc Finger Proteins: From Atomic Contact to Cellular Function. Kluwer Academic/Plenum Publishers: New York, N.Y.

NRC. 1996. Nutrient Requirements of Beef Cattle 7th Revised Ed. National Academy Press: Washington, D.C.

Prasad, A.S. 2007. Zinc: Mechanisms of host defense. J. Nutr. 137:1-5.

Richey, E.J. 1994. Infectious Bovine Rhinotracheitis IBR (Red Nose). University of Florida IFAS Extension. <http://edis.ifas.ufl.edu/VM051> Accessed March 19, 2007.

Sedgmen, B., E.N.T. Meeusen, and S.A. Lofthouse. 2004. Alternative routes of mucosal immunization in large animals. Immunology and Cell Biology. 82:10-16.

Vruwink, K.G., C.L. Keen, and M.E. Gershwin. 1993. The effect of experimental zinc deficiency on development of the immune system. In: S. Cunningham-Rundles (ed). Nutrient Modulation of the Immune Response. Marcel Dekker, New York

Wintergerst, E.S., S. Maggini, and H.H. Dietrich. 2006. Immune-enhancing role of vitamin C and zinc and effect on clinical conditions. Annals of Nutrition and Metabolism 50:85-94.

Table 1. Growth performance and morbidity data for cattle receiving zinc solution as an oral drench, zinc solution as a nasal spray, or no zinc treatment.

	Control	Oral	Nasal	SE	P-value
Initial body weight, kg	229	228	228	3.9	0.96
Final body weight, kg	268	263	256	3.8	0.15
Supplement intake, kg/d	1.08	1.10	1.07	0.024	0.76
Average daily gain, kg (d 1 to 28)	0.92[a]	0.75[a]	0.65[b]	0.065	0.04
Average daily gain, kg (d 1 to 42)	0.93[a]	0.81[a,b]	0.67[b]	0.061	0.06
Morbidity, %	60	72	86	12.3	0.43
Number of pulls	0.7	1.3	1.3	0.25	0.21
Medication cost, $/calf	10.14	18.44	18.50	3.63	0.22

[a,b] $P < 0.10$

Table 2. A list of bacteria cultured from the nasal membrane swabs of cattle treated with zinc solution as an oral drench, zinc solution as a nasal spray, or no zinc solution.

Pasteurella multocida
β- Escherichia coli
Escherichia coli
α- Streptococcus sp.
Staphylococcus sp.
Bacillus sp.
Moracella lacunata
Serratia marcescens
Lactose-E. coli
Pseudomonas aeruginosa
Enterobacter sp.

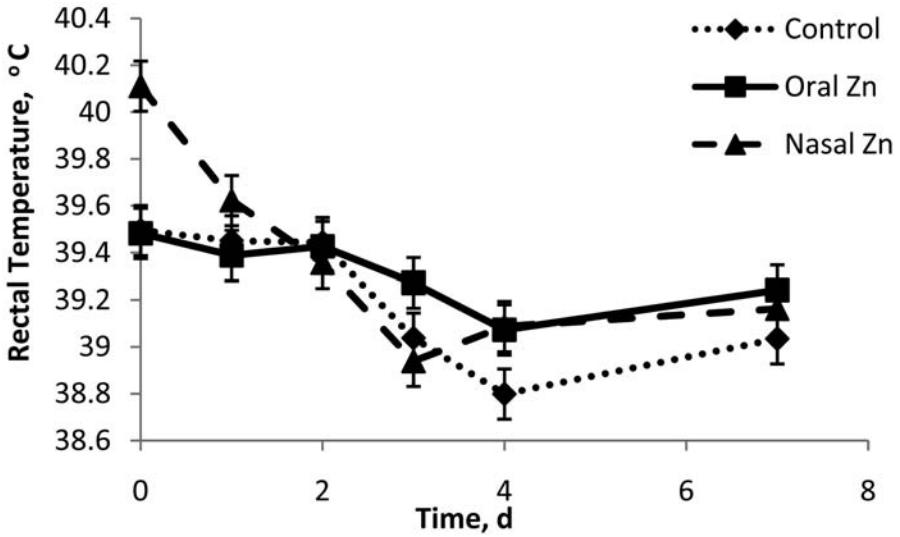

Fig. 1. Average rectal temperatures of cattle receiving no zinc treatment (Control), zinc solution as a drench (Oral), or zinc solution as a nasal spray (Nasal). Treatment by day interaction (P = 0.01).

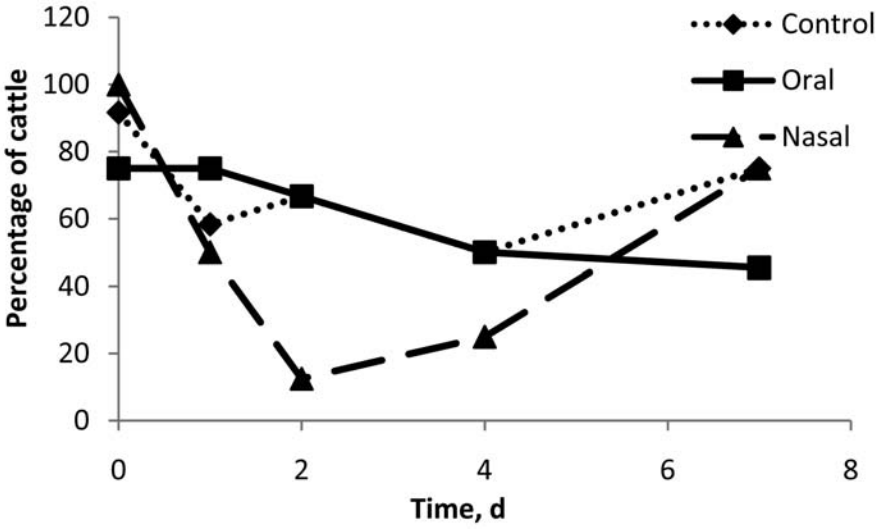

Fig. 2. Percentage of cattle receiving no zinc treatment (Control), zinc solution as a drench (Oral), or zinc solution as a nasal spray (Nasal) with positive nasal membrane swabs for *Pastuerella multocida*. Treatment by day interaction (P = 0.07).

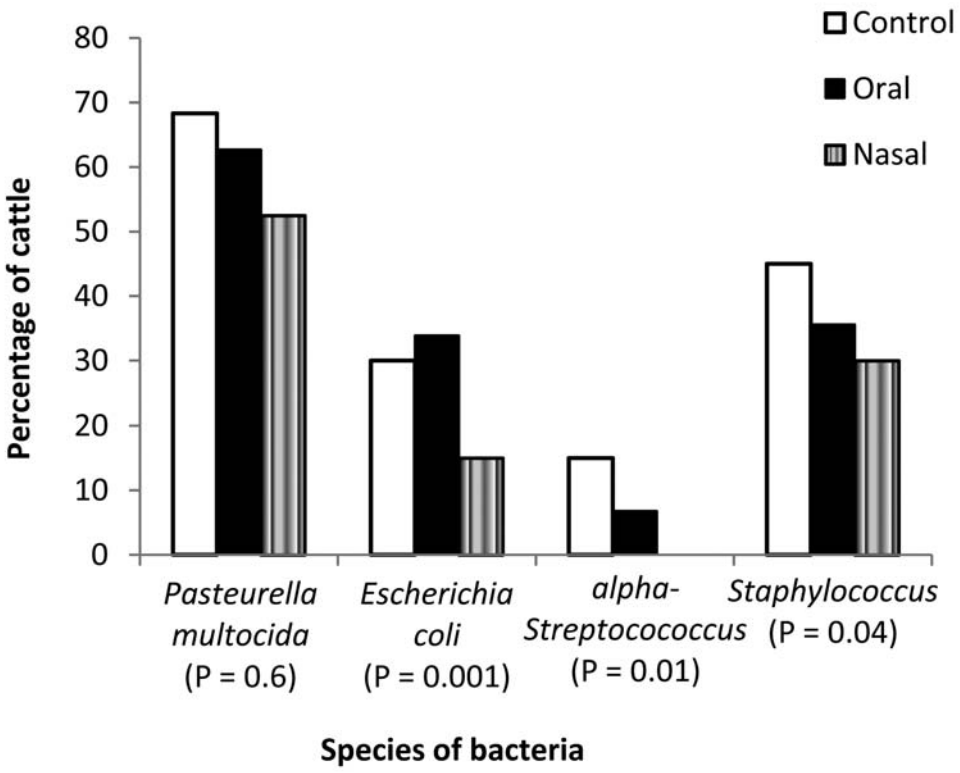

Fig. 3. Percentages of different bacterial species found on nasal membranes swabs of cattle receiving no zinc treatment (Control), zinc solution as a drench (Oral), or zinc solution as a nasal spray (Nasal).

Developing enterprise budgets for sustainable school gardens: Service learning in a global context

Ashley D. Jones and Jennie S. Popp†*

ABSTRACT

Service learning programs are becoming a part of curricula in universities throughout the United States. The University of Arkansas, Fayetteville, (UAF) established a service learning program that targeted the educational, health, social, and agricultural needs of a community. The focus of this research aimed to provide students, faculty, community members, school officials, and students with a template for crop budgets. These crop budgets are used to evaluate the costs and returns of producing multiple crops at a school. Crops produced in a sustainable garden must meet three criteria: 1) have minimal negative environmental impact, 2) provide just-in-time production of quality crops to meet school needs, 3) be solvent, i.e. the garden generates net positive revenue from the sale of crop or provides cost savings by growing crops at the school rather than purchasing them elsewhere. This proposal focuses on developing enterprise budgets for four crops: chili peppers, cabbage, corn, and tomatoes, and an interactive Excel® budgeting tool to evaluate revenues and expenses of crop production. The design of the interactive budgets is to provide a framework that students at UAF can use in their service learning courses when examining the costs and benefits of agriculturally based projects, while also being a functional aid for the recipients of the service learning program.

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MEET THE STUDENT- AUTHOR

Ashley Jones

After I graduated from Lincoln High School in Lincoln, Ark., in 2005, I began my studies in agricultural economics, with a minor in global agriculture, at the University of Arkansas. I was the recipient of the Honors College Academy Scholarship, in addition to other private scholarships provided by the Dale Bumpers College, the Department of Agricultural Economics and Agribusiness, and the Walton Foundation.

I am an active member in the Agriculture Business Club and am currently serving as president. I am also a member of Agricultural and Applied Economics Association Quiz Bowl, Alpha Zeta, Gamma Sigma Delta, and Phi Kappa Phi.

I plan to begin graduate school at Kansas State University in the fall of 2009, to pursue a master's degree in agricultural economics.

This research project was selected because of my involvement with the Belize Service Project in the summers of 2007 and 2008, along with my personal interest in sustainable agriculture in developing countries.

INTRODUCTION

In 2007, the University of Arkansas, Fayetteville, pioneered a new approach to service learning by developing an integrated multi-tasked, multi-year, service-learning program with the objectives of targeting health, educational, agricultural, dietary, social, economic, and environmental needs of a community at the same time. This paper explores the agricultural and food project, a sustainable school garden at St. Matthew's Elementary in Pomona, 10 miles from Dangriga, Belize. The purpose of this research was to create interactive enterprise production budgets for vegetables that could be used in sustainable school gardens in Belize, the U.S., and other countries.

There are several types of budgets, all of which meet different needs and objectives for different users. Basic enterprise budgets give "an estimate of the potential revenue, expenses, and profit for a single enterprise" (Kay, et al., 2004). Budgets can also be modified to fit the user's needs. For vegetable crops, the typical budget is on a per-acre basis. While a paper budget can be useful in computing revenues and expenses for a sample plot, an interactive enterprise budget allows users to modify the budget to fit their needs and compute sensitivity analysis for each crop. Furthermore, interactive budgets that can evaluate multiple crops at one time allow users to determine the most profitable production mix for the planting period. Therefore, the resulting tool serves the dual purpose of being an educational tool for students interested in learning about budgeting and a practical tool for those planning a garden.

This research focused on the development of an interactive four-crop enterprise budget. For purposes of example, the enterprise budgets were used to evaluate the options for a school garden in Belize. The remainder of this paper describes the development of the budget and presents an example that shows how this budget can be used to: 1) calculate revenues and expenses associated with a school garden; 2) evaluate expenses and returns of multiple crops; and 3) determine which combination of crops results in profit maximization, when faced with constraints such as garden area and expenses.

MATERIALS AND METHODS

This research was conducted in three parts. First, through a literature review, information concerning the important parts of small vegetable enterprise budgets was gathered to serve as a model for developing the budgeting tool and to identify the types of data needed for collection in Belize. An enterprise budget includes two main sections: revenues and expenses. The revenue section of a budget includes crop yield and crop prices to generate total revenue. Expenses are segmented into total variable expenses and total fixed expenses, and then totaled to arrive at total expenses for the project. Variable expenses typically include seeds, pesticides, fertilizer, labor, some irrigation expenses, and interest on capital. Fixed expenses typically

include irrigation, machinery and equipment, and management expenses. Relevant expenses were populated for the Belize example; irrelevant expenses were not.

Second, information to populate the budgets was gathered on-site in Belize during summer 2008. Based on local growing conditions and dietary needs, four crops–cabbage, corn, chili peppers, and tomatoes–were identified for use in the budgets. With assistance from the Belizean Ministry of Agriculture, relevant production practices, production inputs, and expected prices and yields were determined.

Third, four paper budgets–one for each crop–were developed that include the collection production practice, yield, and price information. The paper budgets include three main parts: total revenues, total expenses and profit. The following equations were used to calculate profit for each crop:

$$TR = P_y * Y \quad \text{Eq. (1)}$$

$$TE = TVC + TFC \quad \text{Eq. (2)}$$

$$TVC = \sum_{1}^{i} P_{x_i} X_i \quad \text{Eq. (3)}$$

$$TFC = \sum_{1}^{j} P_{w_j} \quad \text{Eq. (4)}$$

$$Profit = TR - TE \quad \text{Eq. (5)}$$

where TR is total revenue, P_y is output price, Y is yield, TE is total expenses, TVC is total variable expenses, TFC is total fixed cost, P_{x_i} Pxthe price for variable input i, X_i is use of variable input i, and P_{w_j} is price of fixed input j.

Fourth, this information was used to create an interactive budget in Excel® that allowed users to compare the inputs, expenses, and revenues for a model garden to that of their own sustainable school garden (Microsoft, 2007). The budget maximizes profits per acre of land, subject to the percentage of total area for each crop. Other constraints include minimum and maximum area allocation for each crop. These constraints are given by a set of formulas identified within the algorithm. Examples of these formulas are:

$$MaxLand = 1 \quad \text{Eq. (6)}$$

$$\%Pep \leq PepMax \quad \text{Eq. (7)}$$

$$\%Pep \geq PepMin \quad \text{Eq. (8)}$$

where *MaxLand* is the maximum land available for production (here one acre), *Pep* is the land devoted to peppers, *PepMax* is the maximum percentage of land that can be allocated to peppers, and *PepMin* is the minimum percentage of land that can be allocated to peppers. Excel® then uses an algorithm to determine the optimal mix of crops that maximizes profits for one acre of land given the identified constraints.

RESULTS AND DISCUSSION

The interactive budget tool is an Excel® 2007 spreadsheet consisting of seven tabs. Four tabs represent the enterprise budgets for each crop. Each budget is comprised of four sections: revenues, variables expenses, fixed expenses, and profits. Variable expenses included: plants and seeds, fertilizers, pesticides, labor, and operating costs. Fixed costs included amortization for irrigation equipment, machinery and other equipment, and management. Users can calculate profit with the default values included in the budgets or they can modify any values to make calculations that are representative of their garden.

The amortization schedule for irrigation was developed in another tab. Information in this tab includes irrigation costs, interest rate, and payment period. The user can adjust all of this information as needed. The input tab of the spreadsheet defines the inner workings of the spreadsheet (such as calculating planting density, yield per area, and output prices) and will not likely be modified by most users.

Finally, the first tab of the spreadsheet is the budget summary. The summary tab displays expenses and returns associated with the chosen mix of crops for production. This tab provides three types of budgeting summaries. The first summary, entitled *Economic Snapshot of My Farm,* provides the revenue, expense, and profit information associated with the user's actual garden situation (Table 1). *My Farm* consists of two acres of production, including 0.65 acres of peppers, 0.45 acres of cabbage, 0.30 acres of corn, and 0.60 acres of tomatoes. Economic returns for these two acres were $12,451. The second summary, *Economic Returns for One Acre of Each Crop*, illustrates revenues, expenses and returns associated with producing one full acre of each crop. Economic returns per acre were as follows: $710 (peppers), $13,449 (cabbage), $1,636 (corn), and $9,078 (tomatoes).

Finally, the third summary shows the results of the optimization process. For example, suppose the owner of *My Farm* wants to produce at least some amount of each crop in his garden. The land constraints would be set as a minimum of 5% and maximum of 85% of the land in one crop. Given default prices, yields, and the amortization schedule, the optimal allocation would be 5% of acreage dedicated to peppers, corn, and tomatoes while cabbage would occupy 85% of the acre. The profit associated with this mix is $12,003 (Table 2). This summary allows the user to conduct sensitivity analysis. For example, if cabbage prices fall from $1.75/lb to $1.05/lb, pepper prices increase from $0.50/lb to $0.90/lb, and yields fall from 10,000 lbs/acre to 7,000 lbs/acre. In this example, profits for *My Farm* fall

from $12,003 to $11,446 and the allocation of crops in the garden move from 85% cabbage to, now, 85% tomato and per-acre profit falls to $8,272 (Table 3).

The budgets created will provide students in the UAF Service Learning Program with a tool to use when analyzing what mix of crops to produce for the upcoming summer in Belize, while also providing agricultural personnel and school faculty a hard copy form of a budget to estimate revenues for the school, as well as expenses for the project.

Sample budgets will be distributed electronically and in hard copy in the UAF Service-Learning Program curriculum, as well as in Belize. The budgets will be capable of being used interactively through Microsoft Excel®, and can be printed and distributed on paper for use where computers are not easily accessible. This research provided an interactive budgeting tool to help UAF students prepare for agricultural projects in Belize, as well as provided the students with a framework that can be used in their service learning course when evaluating the costs and benefits of agriculturally based projects, while at the same time being useful to the recipients of the service learning program, in this example, the schools in Belize.

Once the budgets were developed, a short "how to" guide was developed to help users navigate their way through the spreadsheets to arrive at their own crop revenues and expenses, as well as to provide answers to frequently asked questions. The guide highlights the types of analysis that can be performed and provides instructions on how to perform them.

ACKNOWLEDGEMENTS

Financial support for this project was provided by means of a Student Undergraduate Research Fellowship (SURF), a University of Arkansas Honors College Research Grant, and a Dale Bumpers College of Agricultural, Food and Life Sciences Undergraduate Research grant. I would like to thank my honors thesis committee, Dr. Nilda Burgos, Dr. Jennie Popp, and Dr. Curt Rom for their assistance with this project. I would also like to thank Germán Rodríguez for his assistance in helping design the Excel® budgets.

LITERATURE CITED

Kay, R.D., W.M. Edwards, and P.A. Duffy. 2004. Farm Management. McGraw-Hill, New York.

Microsoft Excel®. 2007. Microsoft Corporation.

Table 1. Economic Snapshot of My Farm.

Economic Snapshot of My Farm—2.00 acres					
	Pepper	Cabbage	Corn	Tomato	
Revenues	$7,150	$7,875	$1,248	$7,500	
Costs	$6,688	$1,823	$757	$2,053	
Profits	$462	$6,052	$491	$5,447	
			Total Profits ($/area)		**$12,451**

Table 2. Optimization of crop mix across one acre of land, given prices, yields, and amortization.

Optimization of 1 Acre of Land - Crop Mix Composition				
	Percentage Area (ac)			
	Pepper	Cabbage	Corn	Tomato
	0.05	**0.85**	**0.05**	**0.05**
	Area Constraints			
Variable	Pepper	Cabbage	Corn	Tomato
At least (ac)	0.05	0.05	0.05	0.05
Maximum (ac)	0.85	0.85	0.85	0.85
Max Area (ac)	1.00			
	Economic Snapshot for Crop Mix ($/1 ac)			
Variable	Pepper	Cabbage	Corn	Tomato
Revenues	$550	$14,875	$208	$625
Costs	$514	$3,443	$126	$171
Profits	$36	$11,432	$82	$454
		Total Profits ($/ac)		**$12,003**

Table 3. Optimization of crop mix across one acre of land when cabbage price and yields fall

Optimization of 1 Acre of Land - Crop Mix Composition				
	Percentage Area (ac)			
	Pepper	Cabbage	Corn	Tomato
	0.05	**0.05**	**0.05**	**0.85**
	Area Constraints			
Variable	Pepper	Cabbage	Corn	Tomato
At least (ac)	0.05	0.05	0.05	0.05
Maximum (ac)	0.85	0.85	0.85	0.85
Max Area (ac)	1.00			
	Economic Snapshot for Crop Mix ($/1 ac)			
Variable	Pepper	Cabbage	Corn	Tomato
Revenues	$550	$14,875	$208	$10,625
Costs	$680	$3,443	$126	$2,909
Profits	($130)	$11,432	$82	$7,716
		Total Profits ($/ac)		**$8,272**

Application of cross-linked rice starches as drug delivery matrices in monolithic tablets

Christopher F. Peluso[*], *Fernanda O. Onofre*[‡], *and Ya-Jane Wang*[‡‡]

ABSTRACT

The sustained release properties of regular and waxy rice starches and their derivatives were studied in tablets. The starches were cross-linked to different levels with epichlorohydrin, and the sustained release properties, swelling power, and rheological characteristics of the matrices prepared were determined. Propranolol hydrochloride was used as a model drug. The sustained release properties of waxy rice starch improved with increasing cross-linking levels, whereas cross-linking had little impact on the functionality of regular rice starch matrices. There was an increase in swelling power for both regular and waxy rice starches as cross-linking levels increased. Both starches showed an increase in the storage modulus when cross-linking was increased, with the regular rice starch matrices showing greater differences among samples. Regular rice starch matrices showed an independence of frequency as cross-linking increased, indicating a "true gel" characteristic, whereas waxy rice matrices behaved as a "weak gel." Both regular and waxy rice starches displayed a decrease in creep and recovery profiles with an increase in cross-linking level. Waxy rice starch showed potential as a sustained release agent in tablets.

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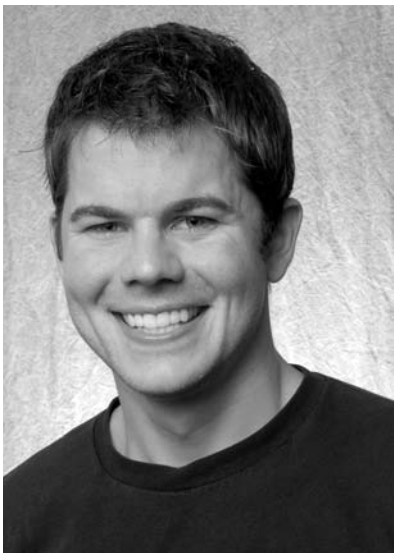
[‡]F. O. Onofre is a Ph.D. candidate in the Department of Food Science.
[‡‡]Y.-J. Wang is the faculty mentor and a professor in the Department of Food Science.

MEET THE STUDENT- AUTHOR



Christopher Peluso

I grew up in Fort Smith and graduated from Southside High School in 2003. I spent two years at the University of Arkansas majoring in physics before moving to New York City to pursue a culinary arts degree. Upon completing culinary school, I worked in kitchens in New York and Florida, returning to the University of Arkansas after 2 years to pursue a degree in food science. I will graduate with a B.S.A in food science in May of 2009. Upon hearing of Dr. Ya-Jane Wang's research in pharmaceutical-based projects, I became interested in one such study involving a new drug delivery matrix for tablets in the medical industry. I have served in Dr. Wang's lab as a laboratory technician and honors research student for the last year. On campus, I have also served as a member of Alpha Phi Omega national service organization, Golden Key Society, and the Food Science Club. Off campus, I am a member of the Institute of Food Technologists and the Washington County Wilderness Search and Rescue Team. I enjoy recreational sports like hiking, caving, and rock climbing. I have enjoyed my time at the University of Arkansas, and particularly everything that the nationally recognized Food Science Department had to offer me. The department treats their students as professional individuals, helping them achieve challenging goals. After graduation, I will be attending medical school at Des Moines University in Iowa, and hope to aid in global medical issues centering on children's health and food issues. I also plan to continue working with professors who have ongoing research in the medical field.

INTRODUCTION

The recent demand and effort to produce safer drugs has led to an emphasis on how drugs are delivered to the human body. Tablets are a common form of drug administration, and different types of drug release systems can exist in a tablet form. For instance, conventional oral delivery systems promote an immediate and complete drug release in the gastrointestinal tract, whereas sustained delivery systems retard the release of the drug from the pharmaceutical form, providing a more constant blood level of the drug over time (Chien, 1989). A common manner of sustaining drug release is by creating a physical barrier through the formation of a gel matrix, which restricts the movement of the drug inside the tablet and its release to the medium. The mechanism of drug release depends on the matrix used, but is commonly associated with drug diffusion through the matrix and matrix erosion and dissolution into the medium.

Acrylic acid derivatives, hydroxypropylmethyl cellulose (HPMC), and starch have been used as tablet excipients. Starch and HPMC are biodegradable and biocompatible, as opposed to acrylic acid derivatives, but starch forms gel faster than HPMC, which may be a great advantage for matrix formation in tablets. Maize starch has been extensively studied in sustained-release systems, but little work has been done on the sustained-release properties of rice starch.

Swelling power, also known as water-holding capacity, corresponds to the ability of a material to hold water. Swelling power is an important property to determine the potential application of a starch polymer matrix as a pharmaceutical excipient in sustained release systems. Upon swelling, water penetrates into the tablets in fronts, which begin on the outside and move inwards until the whole matrix is swollen (Vlachou et al., 2004). Swelling of the resulting tablet in a liquid medium coupled with erosion of the matrix becomes the main component of drug release for a solvent-activated drug delivery system such as a hydrogel (Chien, 1989). It was reported that rice starch in particular would make an adequate tablet filler/drug carrier mainly due to its fine particle size and similar swelling power when compared with other starches like tapioca and potato (Bos et al., 1987).

Rheology studies the viscoelastic properties of matrices and can more thoroughly describe the microstructure of the gel layer in a tablet (Richardson and Kasapis, 1998). Viscoelasticity is an indication of the overall elastic properties of a viscous or semi-viscous material, such as a gel or swollen tablet, and can be characterized by both strain

and recovery capacities of a matrix (Tecante, 2001). Different procedures can be used to evaluate the viscoelastic properties of matrices, including frequency sweep and creep/recovery tests. The frequency sweep test is the application of different twisting movement frequencies on the matrix, and the elastic and viscous components of the system can be measured via the storage modulus (G') and the loss modulus (G"), respectively. The storage modulus is a way of expressing the potential energy stored in a given system temporarily, whereas the loss modulus is the measurement of the amount of energy lost as heat to cause a flow in the fluid matrix. In order to be considered as a gel, the substance must have a storage modulus greater than the loss modulus, among other properties (Jimenez-Avalos et al., 2005). Tan (δ) is the ratio between G" and G', and a reliable way to quantify the relationship between the two moduli. Positive parameters should be obtained for each modulus: tan (δ), creep, and the recovery period (Jimenez-Avalos et al., 2005). In the creep/recovery test, a constant stress is initially applied on the matrix to determine and quantify any deformation that occurs with time. A recovery step follows the creep step, in which the stress is removed and the capacity of the matrix to return to its original state is measured (Jimenez-Avalos et al., 2005). Herman and Remon (1989) reported that the gel strength of hydrated tablets was proportional to the amylose content, with starches lower in amylose having greater gel strength. In addition, the tablets that were low in or free of amylose showed an obstructive gel layer-forming capacity, which was independent of compression force and essential for sustaining the release of a drug from a matrix.

The objectives of this study were: 1) to investigate the effects of starch composition and cross-linking on the sustained-release properties of rice starch tablets, and 2) to characterize and correlate the properties of the matrices with their drug-release abilities.

MATERIALS AND METHODS

Materials. Waxy rice (Remyline AX-DR) and regular rice (Remy DR) starches were obtained from A&B Ingredients, Inc. (Fairfield, N.J.). Propranolol hydrochloride was obtained from TCI America (Portland, Ore.), magnesium stearate from Riedel-de Haën (Seelze, Germany), epichlorohydrin (ECH) from Acros Organics (Morris Plains, N.J.), and sodium sulfate from EMD Chemicals, Inc. (Gibbstown, N.J.). All other chemicals used were of ACS grade.

Cross-linking Reaction. Both waxy and regular rice starches were cross-linked at levels 0.01, 0.05, and 0.1% epichlorohydrin (w/w of starch). Forty grams of starch were combined with 6 g of anhydrous sodium sulfate and 100 mL of deionized water in a 1-L reaction vessel. The pH was adjusted to 12 with 10% NaOH, and the proper amount of ECH was added. The mixture was then covered and stirred at room temperature for 48 h. Afterwards 100 mL of deionized water was added, followed by 50 mL of 20% NaOH to gelatinize the cross-linked starch. The paste was then neutralized to pH 6 with 6 N HCl. The starch was precipitated with 400 mL 85% acetone (v/v), followed by washing 5 × 400 mL 50% ethanol to remove salts, 400 mL 70% acetone, and 400 mL pure acetone. The precipitate was separated into small pieces, dried in a 40°C oven for 2 days, and ground in a Cyclone Sample Mill (UDY Corporation, Fort Collins, Colo.) equipped with a 75-μm sieve. A control of each starch was prepared as described without the cross-linking step. All modified starches and controls were prepared in duplicate.

Preparation of Tablets. A manual mixer (Inversina, Bioengineering AG, Wald, Switzerland) was used to homogenously mix the modified starch (69% w/w) and propranolol hydrochloride (30% w/w) for 10 min. Magnesium stearate was then added (1% w/w) as lubricant, and the mixture was mixed for an additional 1 min. The tablets were prepared by compressing a premeasured amount of 500 mg of the mixture at 2.0 MT using a 13-mm die (Carver, Wabash, Ind.) with a hydraulic press (Carver, Wabash, Ind.).

Drug-release Properties. Drug release properties were studied using a dissolution Apparatus II (paddle) (USP, 2005) instrument (Varian Inc., Cary, N.C.). The medium was 900 mL of deionized water at 37.5°C to simulate human body temperature. The use of the water medium served as a screening process. The tablet was immersed in the water for 24 h using a paddle rotation speed of 50 rpm. Five-mL samples were removed over the course of the time period (0.5, 1, 2, 4, 6, 8, 12, 16, 20, 24 h) with no medium replacement. The concentration of propranolol hydrochloride in the aliquot sample was measured using a spectrophotometer (Beckman Coulter, Fullerton, Calif.) at 290 nm. Triplicate measurements were performed for each starch.

Swelling Power. Swelling power of the modified starches was determined by adding 40 mg of starch sample to 1.5 mL of deionized water in a pre-weighed 2-mL microcentrifuge tube. The tube was vortexed for 5 s, placed in a preheated heat block at 37.5°C for 1 h, and then cooled in an ice-water bath to room temperature. The tube was then centrifuged at 12,000 × g for 10 min, and water was removed from the tube using a dropper. The swelling power was calculated by dividing the weight of the paste by the dry weight of the starch sample. Each starch was measured at least in triplicate.

Rheological Characterization. A tablet was immersed in 5 mL deionized water in a plastic petri dish (3.6 cm dia.), placed in a pre-heated water bath and maintained at 37°C

on a hot plate. The tablet was immersed in the water for 15 min to promote water absorption and swelling of the matrix. The excess water was removed from the petri dish with a disposable pipette and the swollen tablet was placed on the bottom plate of an AR 2000 rheometer (TA Instruments, New Castle, Del.) at 25oC to prevent dehydration. A 40-mm sandblasted plate was used, and a frequency sweep followed by a creep test was performed on each sample. A frequency sweep of 1-100 Hz was performed at 0.2% strain. Four parameters were recorded as a function of frequency: storage modulus (G'), loss modulus (G"), tan δ (G"/G'), and complex viscosity ($|\eta*|$). Upon completion of the frequency test, a creep test was performed. A stress of 1.2 Pa was applied to the tablet, and the compliance (J(t)) was measured for 3 min. The stress was then removed and the recovery was measured for another 3 min. Instantaneous compliance ($J_o(t)$), the slope of the linear region of the compliance curve, and recoverable compliance (the difference between maximum compliance and final compliance) were recorded. All tests were done in six replicates.

Statistical Methods. Standard error and standard deviation were calculated among each type of sample for comparison. The calculations were used to ensure the precision of replicate trials for each concentration of starch tablet.

RESULTS AND DISCUSSION

Drug Release

Cross-linking did not dramatically change the sustained release properties of regular rice starch (Fig. 1A), but affected the rate of drug release from waxy rice starch matrices when compared with the control (Fig. 1B). Cross-linking to a low level (0.01%) did not change the drug-release properties of waxy rice starch, but cross-linking to intermediate (0.05%) and high levels (0.1%) led to a decrease in the rate of drug release. It can also be observed that the overall rate of drug release from waxy rice matrices was significantly lower than that from regular rice matrices.

Tablets made with regular rice starches showed more erosion than those made with waxy rice starches (personal observation). After 24 h, waxy rice tablets usually had a core remaining, while regular rice tablets were completely dissolved in the medium. These remaining cores likely entrapped some drug, accounting for the incomplete drug release observed in waxy rice starches. Overall, the findings of this study showed an improvement in the sustained release properties of waxy rice starch with an increase in the cross-linking level.

Swelling Power

Overall, cross-linking led to an increase in swelling power as the cross-linking level increased for both starch types, although the swelling power of waxy rice cross-linked to the lowest level was similar to the control (Fig. 2).

There was a correlation between the swelling power of waxy rice matrices and their sustained release properties. The sustained-release ability of waxy rice matrices increased with an increase in the cross-linking level, and that was accompanied by an increase in the swelling power of the matrix. This correlation was not very clear for regular rice matrices in this study because all the matrices showed similar drug-release properties.

Rheological Properties

Frequency Sweep Test. In all starches, G' was greater than G" for the frequency sweep test, which was indicative of the predominantly elastic behavior of the matrices (Fig. 3).

The G' values increased as cross-linking increased in regular rice starch (Fig. 3A). On the other hand, the G' values for all cross-linked waxy rice starches were similar and slightly higher than those of the unmodified control, particularly at higher frequencies (Fig. 3B). Cross-linked regular rice starches showed an independence of frequency at lower frequencies, and the 0.1% cross-linked regular rice starch was independent of frequency for a wider range of frequencies. Matrices with less frequency dependence possess "true gel" characteristics (Almdal et al., 1993). This frequency independence was not observed in waxy rice starches, suggesting that amylopectin structure contributes to the frequency dependence in starch gels. Matrices that are more frequency dependent are considered to be "weak gels" or viscous fluids (Clark and Ross-Murphy, 1987). Furthermore, G' values of regular rice starches were higher than those of waxy rice samples, and than their corresponding G" values by around 10×. In waxy rice starches, G' was higher than G", but the difference was smaller than 10×.

Both regular and waxy rice samples showed an increase in tan δ with decreasing frequency (Fig. 4). Regular rice samples (Fig. 4A) showed smaller and more similar tan δ values for different cross-linking degrees, indicating that the changes in both G' and G" for different cross-linking levels were in the same order of magnitude for different frequencies. Meanwhile, for waxy rice starch (Fig. 4B), the control and 0.01% cross-linked starches showed a steep increase in tan δ with a decrease in frequency, indicating a more significant change in G' when compared to G" for the frequencies studied. These results showed that the elastic component of waxy rice samples was more affected by cross-linking than the viscous component. On the other hand, waxy rice starches with higher cross-linking levels did not show a drastic change in tan δ values, indicating a more uniform change in both moduli with frequency.

Overall, the viscosity, $|\eta^*|$, of regular rice matrices was

higher than that of waxy rice ones, showing the importance of amylose and amylopectin to the properties of each starch (Fig. 5). Regular rice starch matrices cross-linked to 0.01 and 0.05% levels and the control showed similar profiles with small variation over different frequencies. However, regular rice starch cross-linked to 0.1% showed a steady increase in viscosity with a decrease in frequency, displaying a shear thinning behavior (Fig. 5A). All waxy rice matrices showed fairly constant complex viscosity profiles throughout the frequency range tested (Fig. 5B). Cross-linking of rice amylopectin did not have a strong impact on the complex viscosity of waxy rice starches.

Overall, the frequency data showed that the G' modulus was lower for starches with lower amylose content. These results imply that the matrices of regular rice starches had a more elastic nature, whereas waxy rice matrices showed a more pronounced, viscous modulus. This more prominent fluidity of waxy rice matrices was supported by the complex viscosity data, which showed lower values for these matrices when compared to those of regular rice.

Creep Test. Figure 6 displays the creep and recovery profiles of all starch matrices, and Table 1 lists the creep-test parameters calculated from the data. Overall, the same trend was observed in both regular (Fig. 6A) and waxy (Fig. 6B) rice starch matrices. Cross-linking led to a drastic decrease in compliance, with starches cross-linked to the highest level showing the lowest compliance. Furthermore, it could be observed that waxy rice matrices showed higher creep and recovery compliance over time when compared to regular rice matrices, indicating the importance of amylose for the behavior of the starch matrices.

From Table 1, it can be observed that cross-linking decreased both the instantaneous compliance and slope of the creep curve for both waxy and regular rice matrices, indicating the formation of a more organized matrix with increasing cross-linking for both starch types. Viscosity increased with increasing cross-linking for both starch types, with higher viscosity values being attributed to the higher amylose content of regular rice in all cases. Furthermore, the recoverable compliance decreased as cross-linking was increased for both starch types, probably as a result of an increase in organization and stiffness of the cross-linked samples. Overall, the two starch types showed the same trends but with different magnitudes.

SUMMARY

In vitro drug release tests showed that waxy rice starch tablets had better sustained- release capacity than regular rice starch, and this ability improved with increasing cross-linking levels. Cross-linking did not have a significant effect on the sustained-release ability of regular rice matrices. Overall, the amylose/amylopectin ratio in rice starch seemed to have played a significant role in the sustained-release properties of rice starch matrices. Similar trends were observed for the effect of cross-linking on the swelling power and rheological properties of both rice starches. Increasing cross-linking levels led to an increase in swelling power and a decrease in elasticity, accompanied by an increase in organization, stiffness, and viscosity of the matrix. In the case of amylopectin-rich waxy rice starch, an increase in viscosity and organization of the matrix was desired to improve its sustained-release properties, which was followed by an increase in the swelling power and water-holding capacity of the matrix, forming a better gel to sustain drug release. On the other hand, the sustained-release properties of amylose-containing rice starch matrices were not strongly affected by cross-linking, and the changes observed by swelling power and rheological characterizations did not result in different drug-release abilities. It is possible that the high amount of amylose in regular rice played a more dominant role in the sustained-release capacity than did the modifications performed in this study.

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LITERATURE CITED

Almdal, K., J. Dyre, S. Hvidt, and O. Kramer. 1993. Towards a phenomenological definition of the term 'gel'. Polymer Gels Networks. 1:5-17.

Bos, C.E., G.K. Bolhuis, H. van Doorne, and C.F. Lerk. 1987. Native starch in tablet
formulations: properties on compaction. Pharmacy World and Science. 9:274-282.

Clark, A.H. and S.B. Ross-Murphy. 1987. Structural and mechanical properties of biopolymer gels. Advances in Polymer Science. 83:57-192.

Chien, Y.W. 1989. Rate-control drug delivery systems: controlled release vs. sustained release. Medical Progress through Technology. 15:21-46.

Herman, J. and J.P. Remon. 1989. Modified starches as hydrophilic matrices for controlled oral delivery. II: In vitro drug release evaluation of thermally modified starches. International Journal of Pharmaceutics. 56:65-70.

Jimenez-Avalos, H.A., E.G. Ramos-Ramirez, and J.A. Salazar-Montoya. 2005. Viscoelastic characterizations of

gum Arabic and maize starch mixture using the Maxwell model. Carbohydrate Polymers. 62:11-18.
Richardson, K. and S. Kasapis. 1998. Rheological methods in characterization of food biopolymers. Development in Food Science. 39:1-49.
Tecante, A. 2001. Food Engineering: Viscoelasticity. Eolss Publishers Co., Inc.: London
Vlachou, M., H. Naseef, and M. Efentakis. 2004. Image analysis studies of dimensional changes in swellable hydrophilic polymer matrices. Polymers for Advanced Technologies, 15:683-689.

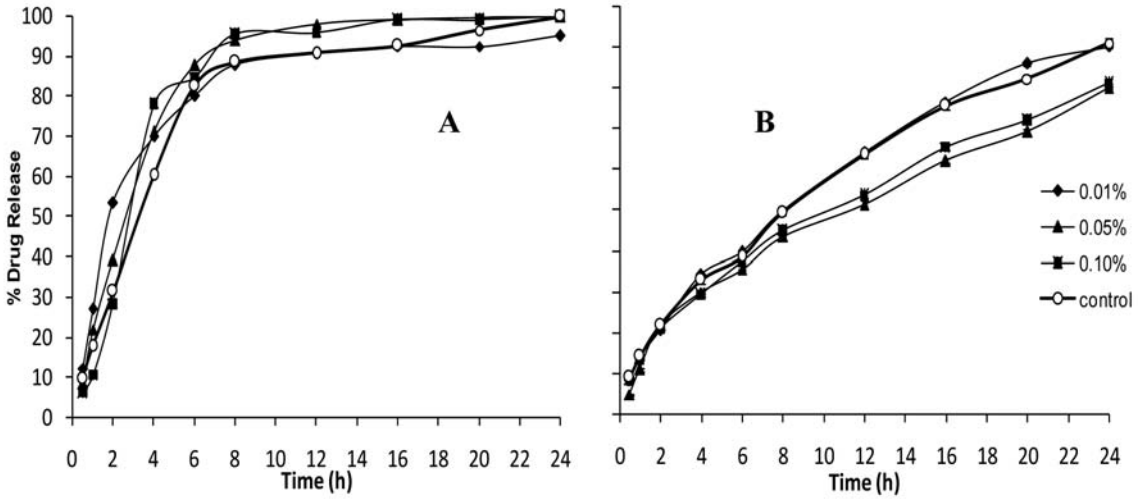

Fig. 1. Drug release profiles from regular rice (A) and waxy rice (B) starches. The percentages are the cross-linking levels prepared.

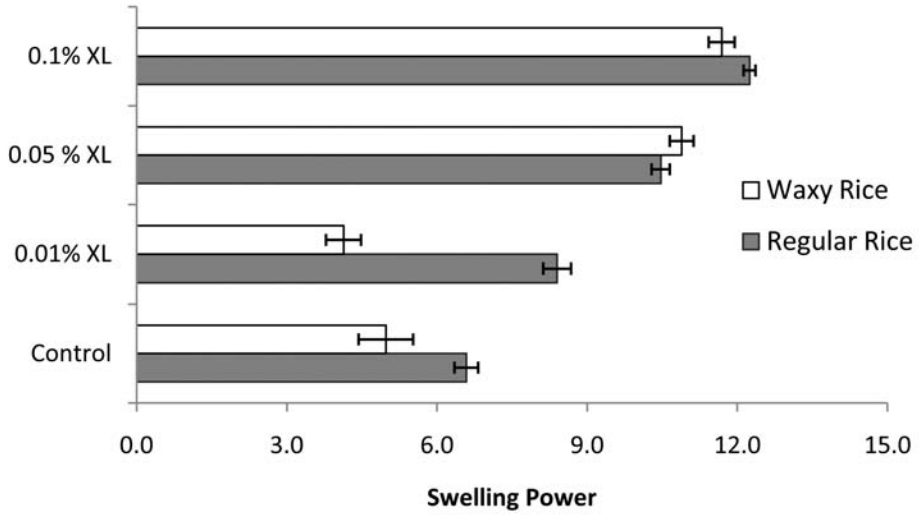

Fig. 2. Swelling power of regular and waxy rice starches. The percentages are the cross-linking levels prepared. The error lines represent the standard error for each cross-linked sample.

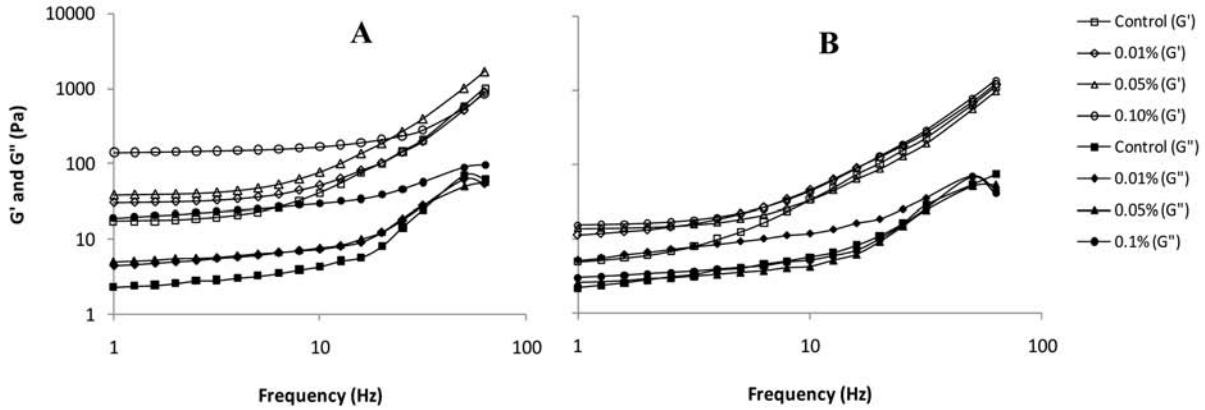

Fig. 3. G' and G" values by frequency for regular (A) and waxy (B) starch matrices. The percentages are the cross-linking levels prepared.

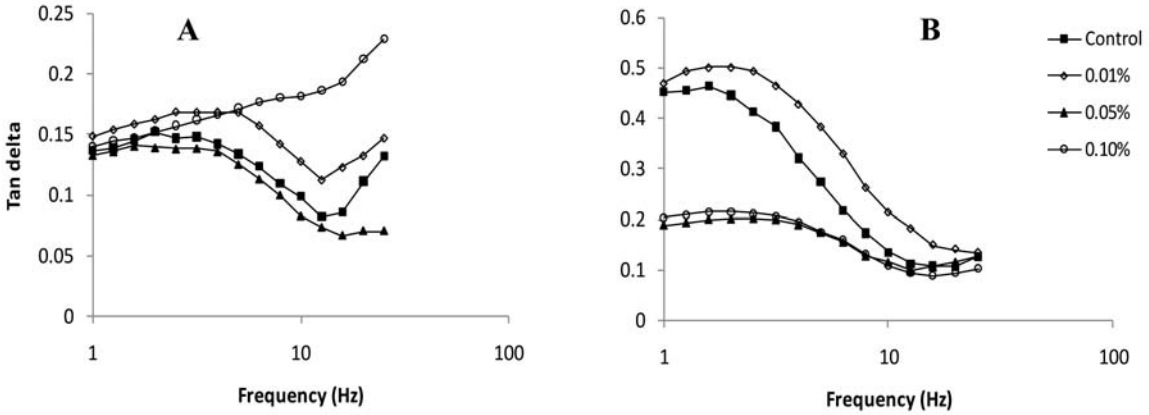

Fig. 4. Tan δ as a function of frequency for regular (A) and waxy (B) rice starch matrices. The percentages are the cross-linking levels prepared.

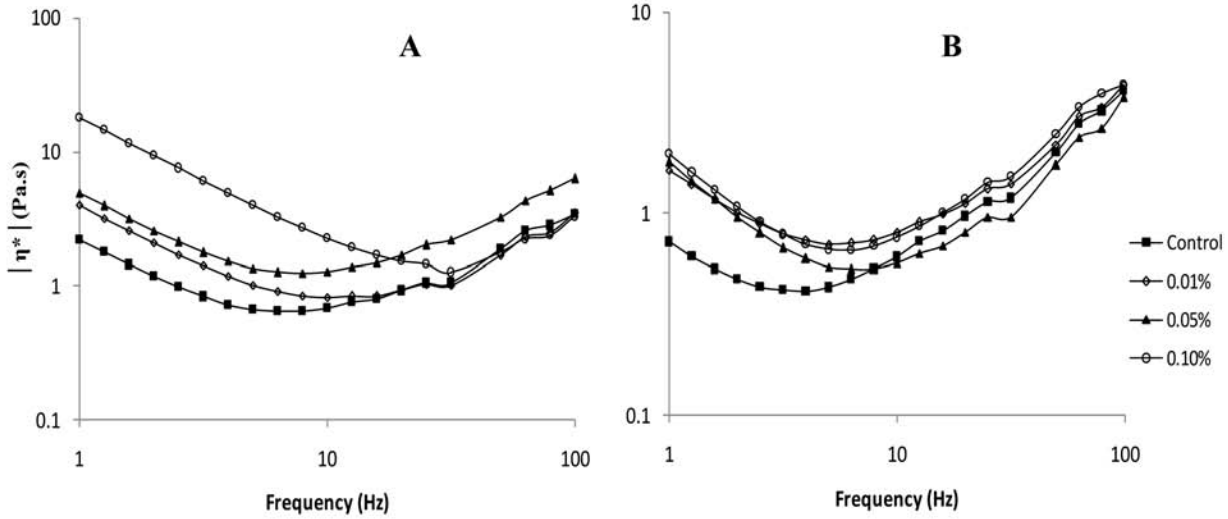

Fig. 5. Complex Viscosity |η*| by frequency for regular (A) and waxy (B) rice starch matrices. The percentages are the cross-linking levels prepared.

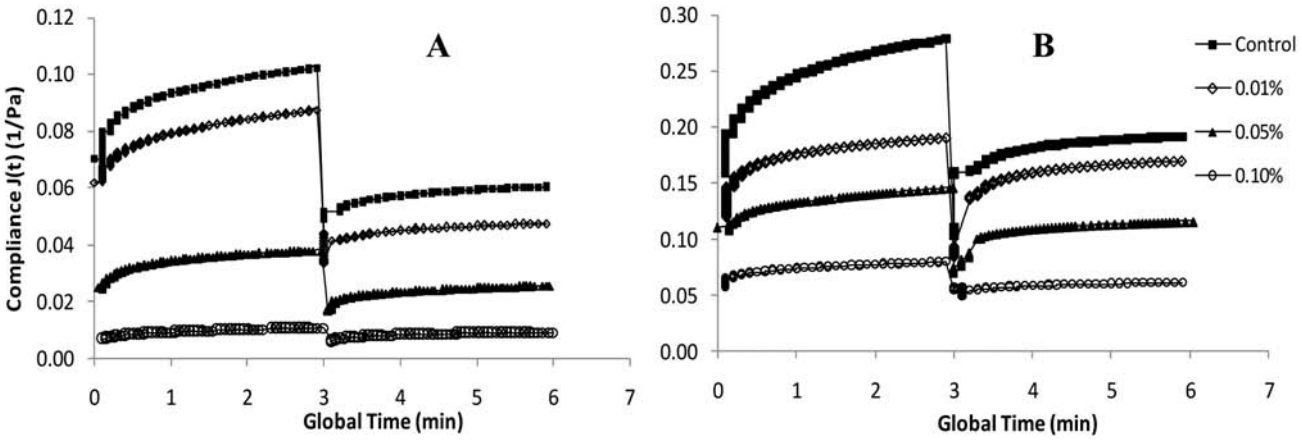

Fig. 6. Creep and recovery profiles of regular (A) and waxy (B) rice starch matrices. The percentages are the cross-linking levels prepared.

Table 1. Creep and recovery parameters of regular and waxy rice starch matrices.

Starch type	Cross-linking level (%)	Instantaneous compliance J_o (Pa)	Slope	Viscosity η_o (Pa.s)	Recoverable compliance (1/Pa)
Regular rice	Control	0.07	0.005	200	0.042
	0.01	0.06	0.005	200	0.040
	0.05	0.03	0.002	500	0.012
	0.1	0.01	0.001	1000	0.002
Waxy rice	Control	0.16	0.017	59	0.088
	0.01	0.12	0.008	125	0.020
	0.05	0.11	0.007	143	0.030
	0.1	0.06	0.004	250	0.019

Measurement of transient smoke emissions from diesel and biodiesel fuel blends in an agricultural tractor

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ABSTRACT

Transient smoke emissions pose potential hazards to human health and the environment. With the increased popularity of biodiesel, there is a need to determine if these fuels produce different levels of particulate matter in exhaust emissions. This study examined the transient smoke emissions of three fuels: No. 2 petroleum diesel fuel (D2, ASTM D 975), a blend of 20% biodiesel and 80% petroleum diesel (B20, ASTM 6751), and a 100% pure biodiesel derived from animal fats (B100, ASTM D 6751). Measurements of smoke emissions were taken using the SAE J1677 snap acceleration test procedure on a John Deere 3203 compact utility tractor. The results indicate there were no statistically significant differences in smoke opacity between the three fuels ($p>0.05$). The low, non-significant emissions may be due to the diesel engine being EPA Tier II-compliant and the use of ultra-low-sulfur diesel. Recommendations for further study include testing biofuels made of varying feed stocks rather than animal fats, testing steady state load conditions in addition to transient loads, and testing tractors manufactured prior to initiation of EPA tier-compliance standards.

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MEET THE STUDENT- AUTHOR

Kristin Pennington

I am a native of Rogers, Ark., and graduated from Rogers High School in 2005. I transferred to the University of Arkansas as a junior agricultural education major and later declared a minor in agricultural systems technology management. I have been active in multiple student clubs including Ag Mech, AEED Reps, and Collegiate FFA/4-H. I am also a member of Alpha Zeta and Gamma Sigma Delta. I serve on the Bumpers College Student Advisory Board. In 2007-2008, I was honored as the Distinguished Transfer Scholar for the college. Upon completion of my undergraduate degree, I plan to further my education at the University of Arkansas and pursue a master's degree in agricultural education.

I would like to give a special thanks to Dr. Donald Johnson, the mentor for this project, for his guidance and patience throughout this project. Also, I would like to thank all the faculty and staff of the Agricultural and Extension Education Department for their support and encouragement.

INTRODUCTION

Transient smoke emissions pose potential hazards to human health and the environment. According to U.S. Environmental Protection Agency (2007),

> Particulate matter (PM) is a complex mixture of extremely small particles and liquid droplets in the air. Particulate matter causes concern because it is associated with serious health effects such as aggravated asthma, difficulty breathing, chronic bronchitis, decreased lung function, and premature death. PM contributes to haze and can harm the environment by changing the natural nutrient and chemical balance of the soil (p.1).

Approximately one-fourth of the particle mass inhaled by humans accumulates in the pulmonary region, some of which is retained with a half-life of several hundred days (Glancey et al., 2007).

From 2004 to 2005 alone, production of biodiesel in the U.S. increased from 97 to 291 million liters, a threefold increase (National Biodiesel Board, 2006). Biodiesel is typically blended with petroleum diesel and the percent biodiesel in the blend is designated as BXX, where XX is the percent of biodiesel (e.g. B20 is 20% biodiesel and 80% petroleum diesel). With the increased popularity of biodiesel, and in an effort to lessen emission problems, there is a need to determine if these fuels produce different levels of particulate emissions. The purpose of this study was to compare the particulate matter emissions in a compression ignition engine fueled by petroleum diesel (D2) versus biofuels (B20 & B100).

MATERIALS AND METHODS

This study used an experimental design to find average smoke opacity of an agricultural tractor fueled with three fuel blends. Opacity is a direct indicator of the level of particulate matter in the exhaust stream from a given engine.

Fuels were tested in a John Deere (Moline, Illinois) 3202 three-cylinder, four-stroke, naturally aspirated, compression-ignition, compact utility tractor with a rated engine power of 23.9kW at 2800 rpm and a compression ratio of 19:1. The engine displacement was 1.5 L with an 84 mm bore and 90 mm stroke. The three fuels tested were: D2 (ASTM D 975); B20 (ASTM 6751); B100 (ASTM D 6751) (Table 1).

An Autologic® (Sussex, Wis.) SAE J1667- compliant opacity meter was used to measure opacity. Before running tests, the opacity meter was properly calibrated according to the operation manual. Emission characteristics were quantified by measuring the opacity of the emission gases using the Snap Acceleration Smoke Test Procedure for Heavy-Duty Powered Vehicles (SAE, 1996). Opacity is measured by the percent of light that can pass through the exhaust. If the light passes through the meter with no deflection, the opacity is 0%. Light deflection is due to the amount of particulate matter in the exhaust. The test pro-

cedure consisted of three phases, each held for five (5) seconds. The three phases are warm-up, idle, and maximum governed speed. Each test run consisted of three clean-out trials followed by three recorded trials. When switching between fuels, the fuel tank was drained and the fuel lines were flushed to avoid contamination of fuel samples. Four replications were conducted for each of the three fuels (D2, B20, B100). Data were analyzed using one-way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Petroleum diesel and biofuels were analyzed to display their effects on opacity in the test vehicle. Data analysis showed no significant differences ($p \geq 0.05$) among opacity values of the tested fuel types. The *F* value for analysis of tested fuels was 0.60 with a $p = 0.57$. All opacity values were less than 1% by differentiated fuel types (Table 2).

Having no significant differences among means and opacity values less than 1% in all fuel combinations gives little reason for concern about particulate emissions in this type of engine. However, it should be noted that this test was run with a modern, EPA Tier II-compliant engine. Tier II-and-above compliant engines are designed to produce fewer particulate emissions. Since there are many tractors still in use that were manufactured prior to EPA tier requirements (pre- 1996), which are common in agriculture, further research on tractors that were produced before the Tier I-IV system was adopted should be conducted. Additionally, the D2 utilized in this test was "ultra-low" sulfur diesel (<15 ppm), which produces less emissions than older petroleum- based fuels (Walsh, 2004).

Future studies may include testing biofuels made of varying feedstock's, rather than animal fats, and/or testing steady-state load conditions in addition to transient-load conditions. Varying load conditions may produce combustion characteristics that could produce a wider range of particulate emissions.

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LITERATURE CITED

Glancey, J., A. Chirnside, J. Hummel, S. Nobels, S. Chanpimol and A. Raval. 2007. Measurement of transient smoke emissions characteristics from E-Diesel and Soy-Diesel fuel blends in two commercial engines. ASABE Paper No.076093. St. Joseph, Mich.: ASABE.

National Biodiesel Board. 2006. Biodiesel Fact Sheets: Biodiesel 2006 Backgrounder. NBB, 3337a Emerald Lane, PO Box 104898, Jefferson City, Mo. 65110-4889.

Society of Automotive Engineers. 1996. Snap-Acceleration Smoke Test Procedure for Heavy Duty Diesel Powered Vehicles. Society of Automotive Engineers Standard J1667. SAE World Headquarters. 400 Commonwealth Dr, Warrendale, Penn. 15096-0001 USA.

U.S. EPA. National Center for Environmental Assessment. 2007. Particulate Matter. U.S Environmental Protection Agency. <http://epa.gov> Accessed March 30, 2009.

Walsh, M. 2004. The impact of fuel parameters on vehicle emissions. Proceedings from the 5th China/Asia clean fuels international conference; 2004 Nov 9; Beijing, China.

Table 1. Analytical data for each fuel type.

Tested fuel	$D2_a$	$B20_a$	$B100_b$
Heat of combustion	45.1	44.2	39.8
Cloud point	-14.9	-7.7	-1
Specific gravity	0.8414	0.8485	0.886
Sulfur (ppm)	6.6	6.9	NA
Free glycerin (%)	-[c]	-[c]	-[d]
Total glycerin (%)	NA	.025	NA
Iodine number	NA	14.3	NA
Viscosity (CS@40°C)	2.6	2.9	NA

-[a] Samples of D2 and B20 used in this study were tested by the Future Fuel Chemical Company Laboratory as part of a related project.
-[b] National Renewable Energy Laboratory Liquid Fuel Database.
-[c] Free glycerin not present in petroleum diesel.
-[d] Amounts too small to detect.

Table 2. Exhaust opacity percentages by fuel type.

Fuel Type	*Mean*	*Standard Deviation*
D2	0.94	0.57
B20	0.62	0.50
B100	0.56	0.40

Table 3. Opacity of Fuel Types.

Fuel Type	*Mean*	*Standard Deviation*
D2	0.94	0.57
B20	0.62	0.50
B100	0.56	0.40

Correlation of calcium and magnesium intakes to frequency of muscle cramps in female college athletes

Jennifer Schneider[*] *and Marjorie Fitch-Hilgenberg*[†]

ABSTRACT

Muscle cramps are involuntary, painful, sudden contractions of skeletal muscles that can cause detrimental effects on athletic performance. Recent data suggest that low intakes of dietary calcium (Ca) and magnesium (Mg) can enhance or cause muscle cramps. The purpose of this study was to determine the correlation between Ca and Mg intakes and muscle cramping in female college athletes. Athletes completed a 24-hour dietary recall and a survey on frequency and location of muscle cramps. Of those surveyed, 8 participated in basketball, 21 in softball, and 10 in gymnastics. Calcium and Mg intakes were calculated from dietary recall data using Food Processor® nutrient analysis software. Data are reported as means ± standard deviation (SD). T-tests were performed to determine significant differences between groups. The average daily intakes of Ca above and below the dietary reference intake (DRI) were 1516 ± 559 mg/day and 504 ± 296 mg/day, respectively, for the athletes that cramped and 1620 ± 299 mg/day and 645 ± 250 mg/day, respectively, for the athletes that did not experience cramping. The average daily intakes above and below the DRI for Mg in the cramping group were 423 ± 103 mg/day and 180 ± 65 mg/day, respectively. The average daily intakes for those consuming Mg above and below the DRI in the non-cramping group were 476 ± 80 mg/day and 190 ± 64 mg/day, respectively. No significant differences in the intakes of Ca and Mg were found between groups that cramped and those that did not report cramping. The results of this study suggest that a high intake of Ca coupled with a low intake of Mg is positively correlated to muscle cramping.

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MEET THE STUDENT- AUTHOR

Jennifer Schneider

I am currently a senior in the School of Human Environmental Sciences pursuing a B.S. degree in food, human nutrition, and hospitality with a concentration in dietetics. I am also completing a minor in biology and have completed the University of Arkansas' pre-medical program. I have been a part of various organizations, such as The Student Dietetics Association and Dale Bumpers Student Ambassadors, and have been employed by the University as a fitness instructor. I have enjoyed my time in the honors program because it has given me many opportunities, including a study abroad trip to Italy and research in my desired area, which I could not have received elsewhere. After I graduate, I plan to complete my dietetic internship and continue my education in medical school. Someday, I hope to incorporate principles of nutrition into more medical treatments.

INTRODUCTION

Muscle cramps are involuntary, painful skeletal muscle contractions that can occur in people in a normal or diseased state (Parisi, et al., 2003). Muscle cramps have been associated with many causes including hydration status, electrolyte imbalance, toxins, nerve and tissue damage, and disease. The focus of this study was to determine the effect of calcium (Ca) and magnesium (Mg) intakes on muscle cramping.

Calcium has been the focus of many bone-related ailments, but is often overlooked when analyzing its effect in muscle cramping. Calcium is a major contributor to muscle contraction due to its ionic stat. Calcium is found in many foods including milk products, certain seafood, legumes, and cruciferous vegetables. The majority of adults absorb 20%-50% of the ingested Ca, but the percentage of absorption varies greatly with the intake of other foods or supplements. Vitamin D and some carbohydrates have been found to increase the absorption of Ca. However, phytates and oxalates alter the form of Ca so that it is unable to be absorbed by the human body. Sodium and excess fatty acids have also been shown to decrease Ca absorption (Lopez, et al., 2002).

Magnesium is the fourth most plentiful cation found in the human body (Bilbey and Prabhakaran, 1996) and functions in many nutritional and biochemical reactions. Magnesium is especially important in maintaining necessary electrical gradients between muscle and nerve cell membranes (Maughan, 1999). Magnesium plays key roles in muscle contractions and electrical gradient maintenance. Magnesium levels in the body are easily altered. Serum levels are often lowered by excessive sweating (Bilbey and Prabhakaran, 1996) and by high intakes of phytic acid and fiber (Lopez, et al., 2002). However, there is evidence indicating that carbohydrates, especially fructose, help increase Mg serum levels when Mg intake is either too low or too high to maintain normal balance (Milne and Nielson, 2000). Magnesium is found in a variety of foods, with the richest sources being green leafy vegetables, some seafood, nuts, legumes, and whole-grains. Despite the abundance of Mg in the food supply, only about 40% is normally absorbed in adults with adequate intakes (Bilbey and Prabhakaran, 1996).

Calcium and Mg play complementary roles in muscle contractibility**.** Individual muscle fibers contain two types of filaments, myosin and actin. When separated, these filaments create muscle relaxation and, when united, create muscle contraction. In order for the fibers to come together, Ca must be present to help activate the actin filament, troponin C, so that myosin can bind with it. Magnesium has the potential to act as an antagonist to Ca because it can compete for the same muscle binding sites as Ca at the troponin C location (Landon and Young, 1993). When there is an abundance of Mg in the body, it will take the place of Ca and inhibit a contraction. Conversely, when there is an excess of Ca and a minimal amount of Mg, there is an increased chance of muscle contraction.

The purpose of this study was to determine the relationship between Ca and Mg intakes and muscle cramping in female college athletes. Current research proposed 3hypotheses that will be tested: low intakes of Ca will show a positive correlation with muscle cramping; low intakes of Mg will show a positive correlation with muscle cramping; high intakes of Ca accompanied by low intakes of Mg will positive correlate with muscle cramps. College athletes were chosen as subjects for the study because of the combination of physical and mental strain their bodies endure. Low blood levels of Ca and Mg may be related to muscular cramping (Parisi et al., 2003).

METHODS AND MATERIALS

Research protocol was submitted to the Institutional Review Board at the University of Arkansas. Approval was granted before any data were collected.

Collection of Data. The head coaches of six female college athletic teams were contacted and informed about the research project by telephone and e-mail. The teams consisted of softball, track and field, soccer, gymnastics, basketball, and swimming. Of the six teams, three participated in the survey: basketball, softball, and gymnastics. The surveys were distributed either directly to the team or given to the coach. The basketball team received direct instruction from the researcher in how to complete the survey; whereas, instructions were given to the softball and gymnastics coaches who administered the surveys. The survey requested information on each athlete's sport, food allergies, medications, frequency of exercise per week, frequency and location of muscle cramps, frequency of menstrual cramps, height, weight, and age. An informed-consent form from each participant was completed before surveys were administered. Participants completed a 24-h food intake form to document food intakes. All parts of the survey had to contain complete information for the survey to be considered valid. Each survey was given a letter code to designate sport: BA for basketball, SB for softball, and GM for gymnastics. A number was assigned to each survey to assure confidentiality. The basketball team submitted 12 surveys with 4 discarded because of missing data. The softball team submitted 23 surveys of which 2 were discarded due to missing data. The gymnastics team submitted 10 completed surveys.

Recording and Analyzing Data. Height, weight, gender, activity level, and information from the surveys were entered into Food Processor® Nutrition Analysis Software from ESHA Research, Salem, Oregon to determine individual mineral requirements and mineral intakes. Food Processor® software was used because of its accuracy, extensive food and nutrient database, and means of data export (McCullough, et al., 1999). All survey and mineral intake data were entered into Microsoft Excel® for statistical analysis. Due to differing heights, weights, and ages of the athletes, ESHA Food Processor estimated varying recommended intakes of Ca and Mg for individual athletes. One thousand mg/day of Ca and 310 mg/day of Mg, the dietary reference intakes (DRI) for the minerals, (Yates and Schlicker, 1998), were used as the standard intakes to determine high and low intakes of these minerals. . Data were reported as mean ± standard deviation (SD). The student's t-test was used to determine level of statistical significance between the groups who cramped and the group that did not cramp.

RESULTS AND DISCUSSION

No significant differences in intakes of Ca or Mg were found among the athletes that reported cramping and those who did not report cramping. Data were combined for intakes above the DRI and below the DRI. Results of this study rejected the hypothesis that a low intake of Ca would be positively correlated with cramping. The average intakes of Ca and Mg above and below the DRI are shown in Table 1. In the cramping group, the mean Ca intakes above and below the DRI were 1516 ± 559 mg/day and 504 ± 296 mg/day, respectively. However, the average intake of Ca in athletes that did not experience cramping above and below the recommended amount was 1620 ± 299 and 645 ± 250 mg/day, respectively. The intakes Ca and Mg of the group experiencing cramps were lower in both the high and low intake categories compared to the non-cramping group; however, the differences were not statistically significant. Results did not support the first hypothesis.

Data from this study also reject the second hypothesis that a low intake of Mg is correlated with muscle cramps. The average intake of Mg in athletes that experienced cramping above and below the recommended amount was 423 ± 103 and 180 ± 65 mg/day, respectively. The average Mg intake above the dietary reference intake was 476 ± 80 mg/day and below recommended levels was 190 ± 64 mg/day. These results were similar to those obtained for Ca. However, the differences between the averages for the two groups were not statistically significant (Table 1). The results do not support the second hypothesis that a low intake of Mg is linked with muscle cramping.

The average intakes of Ca and Mg in the cramping and non-cramping groups did not differ significantly. However, results suggest a correlation between athletes with a high Ca intake coupled with a low Mg intake with muscle cramping. Seven athletes that experienced cramps were found to have an intake of Ca above the recommended amount. Four of these 7 athletes or 57% had an intake below the recommended amount of Mg. This finding sup-

ports a possible relationship between high Ca levels and low Mg levels with muscle cramping. However, further investigation is needed to determine the validity of these results.

Data collection and analysis could have been more complete. Diet recall data were often incomplete when participants failed to describe serving sizes, methods of preparation, and brands of the foods consumed. In order to enter data as correctly as possible, similar foods that were not specifically defined by the athlete were recorded as commonly consumed foods. For example, all entries from the athletes that only stated "milk" were recorded as "one cup of 2% milk" for each of those athletes. If "pizza" was entered in the survey, Tony's® 6-inch cheese pizza was used to determine nutritional values. This was done to eliminate any bias and to standardize terminology among incomplete inputs from the athletes. Depending on the participant, this method may have resulted in either over- or underestimating of intakes.

Twenty-four-hour recalls from memory often do not represent the true, normal eating habits of a person. It is likely that some of the participants under or over consumed on the day that the survey was completed. Lack of instruction may have had an impact on participants' understanding of the survey and dietary recall. It is important to have on-site instruction and inspection of the completed survey to assure the collection of accuracy, comprehensive data. Further research should include food diaries, food frequency records, blood mineral tests, and standardized data collection to confirm the results of this study.

Results from this study should not be considered conclusive. More participants need to be recruited and different analytical techniques should be implemented.

LITERATURE CITED

Bilbey, D. and V. Prabhakaran. 1996. Muscle cramps and magnesium deficiency: Case report. Canadian Family Physician. 12:1348-1351.

Landon, R. and E.A. Young. 1993. Role of magnesium in regulation of lung function. J. Amer. Diet. Assoc. 93:674-677.

Lopez, W. H., F. Leenhardt, C. Coudray and C. Remesy. 2002. Minerals and phytic acid interactions: Is it a real problem for human consumption?. International J. Food Sci. Tech. 37:727-739.

Maughan , R. J. 1999. Role of micronutrients in sport and physical activity. British Medical Bull. 55. 683-690.

McCullough, M., N.M. Karanja, P. Lin, E. Obarzanek, K.M. Phillips, R. Laws, W.M. Vollmer, E.A. O'Connor, C. Champagne and M.M. Windhauser. 1999. Comparison of 4 nutrient databases with chemical composition data from the dietary approaches to stop hypertension trial. J. Amer. Diet. Assoc. 99:45-53.

Milne, D. B. and F.H. Nielsen. 2000. The interaction between dietary fructose and magnesium adversely affects macromineral homeostasis in men. J. Amer. College Nutr. 19:31-37.

Parisi, L., F. Pierelli, G. Amabile, G. Valente, E. Caladriello, F. Fattapposta, P. Rossi and M. Serrao. 2003. Muscular cramps: Proposals for a new classification. Acta Neurologica Scan. 107:176-186.

Schwellnus, M. P., N. Drew and M. Coolins. 2008. Muscle cramping in athletes-risk Factors, Clinical Assessment and Management. Clin. in Sports Med. 27:183-194.

Schwellnus, M. P., J. Nicol and T.D. Noakes. 2004. Serum electrolyte concentrations and hydration status are not associated with exercise associated muscle cramping (EAMC) in distance runners. Brit. J. Sports Med. 38:488-92.

Yates, A. A. and S.A. Schlicker. 1998. Dietary reference intakes: The new basis for recommendations for calcium, and related nutrients, B vitamins, and choline. J. Amer. Diet. Assoc. 98:699-706.

Table 1. Consumption above and below the dietary reference intakes (DRI)[1] for calcium and magnesium by female athletes with and without muscle cramping.

Mineral	Cramping	Non-Cramping
Calcium	(mg/day)	(mg/day)
Above DRI	1516 ± 559[2,a]	1620 ± 299[a]
Below DRI	504 ± 296[a,b]	644.65 ± 250[a,b]
Magnesium	(mg/day)	(mg/day)
Above DRI	423 ± 103[c]	476 ± 80[c]
Below DRI	180 ± 65[c,d]	190 ± 64[c,d]

[1] DRI for calcium and magnesium is 1000 mg and 310 mg/day, respectively.
[2] Data reported as group means ± SD.
[a-d] Means followed by the same letter superscript are not significantly different at $P \leq 0.05$.

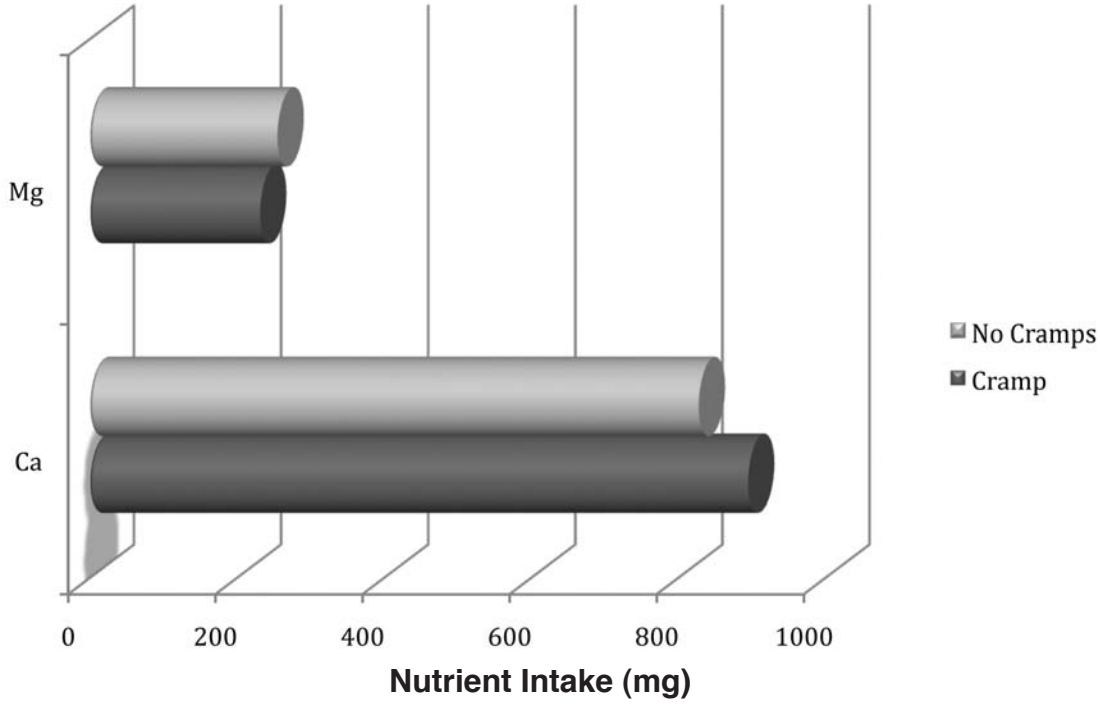

Fig. 1. Overall reported cramping and average nutrient intake.

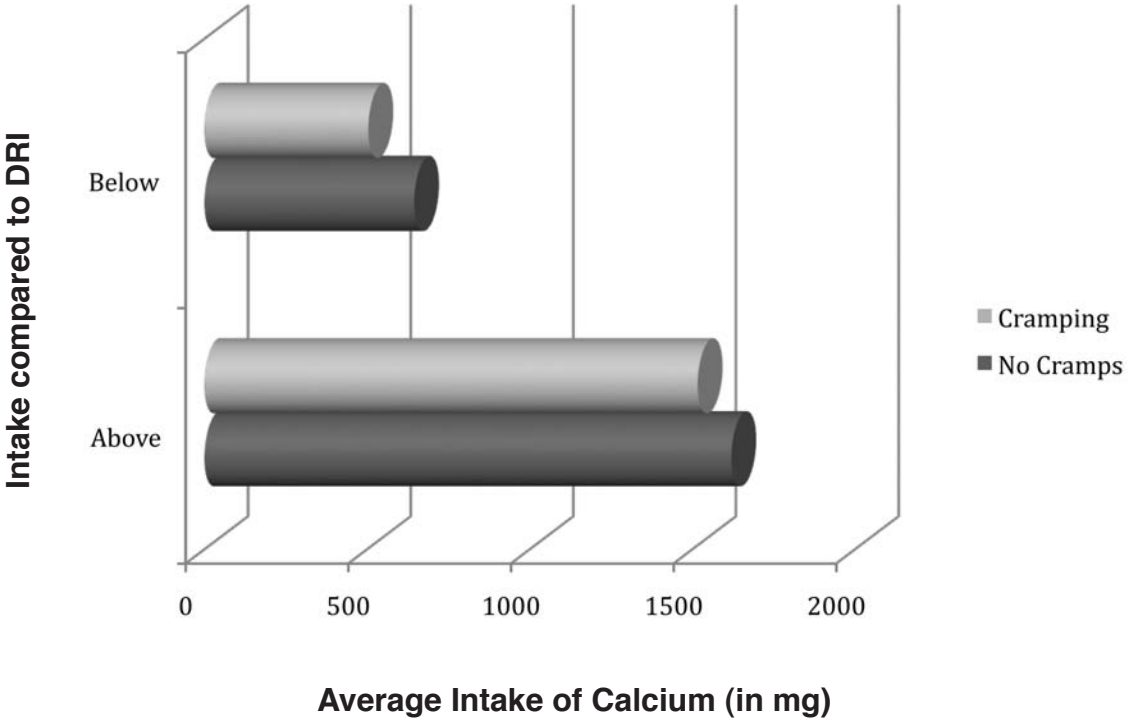

Fig. 2. Average calcium intakes among athletes above and below DRI.

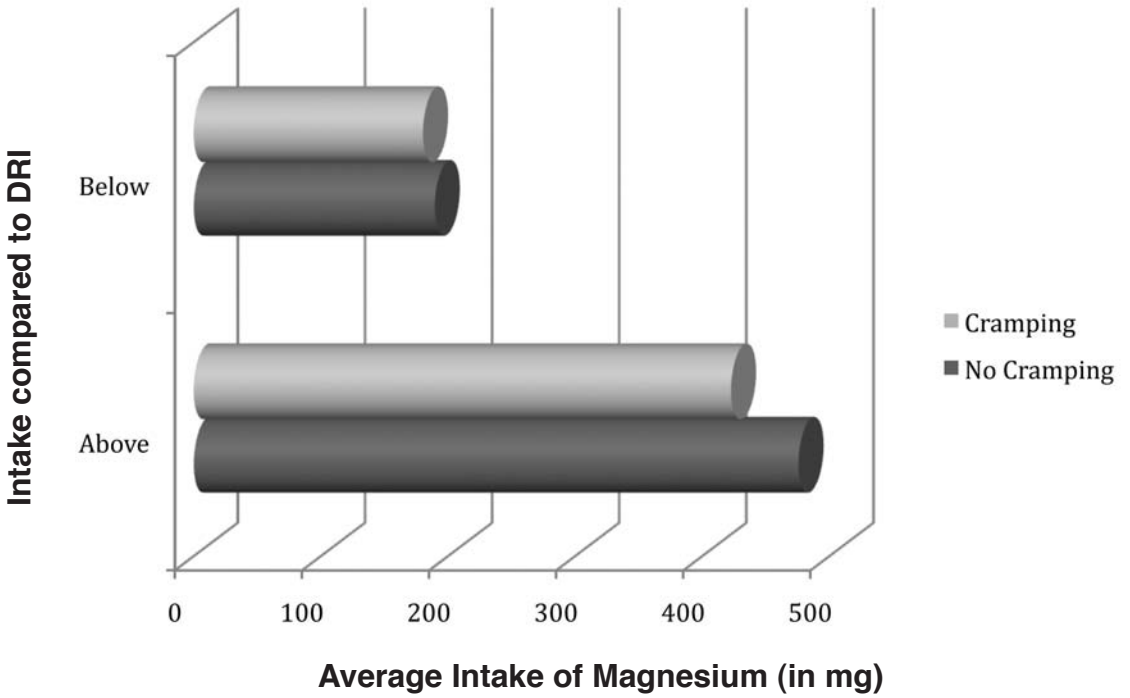

Fig. 3. Average magnesium intakes among athletes above and below DRI.

E-85 vs. regular gasoline: effects on engine performance, fuel efficiency, and exhaust emissions

Jordan W. Steinhaus[*], *Donald M. Johnson*[†], *George W. Wardlow*[§]

ABSTRACT

This study compared the performance, fuel efficiency, and exhaust emissions of a 2.61 kW engine fueled with regular unleaded gasoline (87 octane) and an 85% ethanol blend (E85) under two load conditions. Four 1-h tests were conducted with each fuel at both governor's maximum (3400 rpm) and peak torque (2800 rpm) conditions for a total of 16 tests. At governor's maximum engine speed, there were no significant differences ($p>0.05$) between fuels for engine torque, power, specific carbon dioxide (sCO_2), specific carbon monoxide (sCO), specific hydrocarbons (sHC), or specific oxides of nitrogen (sNO_X) emissions. However, there was a significant difference in specific fuel consumption and specific dioxide (sO_2) emissions with E85 requiring the consumption of more fuel and emitting fewer oxide gases. Under peak-torque test conditions, there were significant differences by fuel for power, torque, and specific fuel consumption, as ethanol required more fuel while developing less power and torque when compared to gasoline. There were no significant differences by fuel type in sCO_2, sCO, sHC, sO_2, or sNO_X emissions. The results indicate that performance was similar when the engine was fueled by regular unleaded gasoline or E85 under rated engine-speed conditions; however, the ethanol-fueled engine produced significantly less power and torque under peak torque testing conditions. In both testing conditions, specific fuel consumption was significantly higher with E85.

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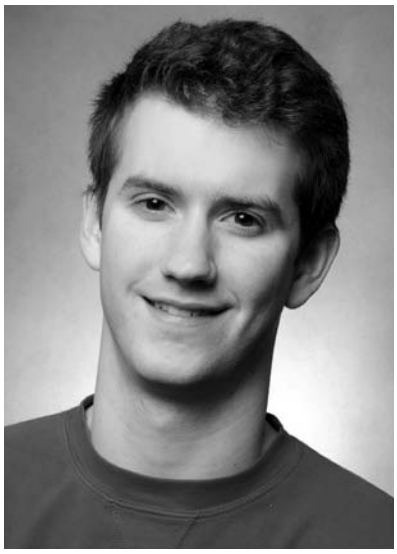

Jordan Steinhaus

I graduated from Lakeside High School in Hot Springs in 2005. In the fall of 2005, I began my undergraduate studies at the University of Arkansas through the Honors College Fellowship, Governor's Distinguished Scholarship, and the Robert C. Byrd Scholarship. During my time at the University, I have been involved with the Honors College, the First Year Experience office through ROCK Camp, Alpha Tau Alpha Honors Fraternity, the Agricultural Mechanization club as Vice President, and Lambda Chi Alpha fraternity. I am a senior majoring in agricultural systems technology management under the direction of Dr. Donald M. Johnson. I was drawn to the ASTM program due to the hands-on involvement of both the professors and their curriculum.

I began working with Dr. Donald Johnson in the agricultural and extension education department to formulate a research plan in the fall of 2007. After receiving funding for this project, we began in the spring of 2008 and have been busy ever since. I thank Dr. Johnson for his support and guidance through this past year and for the opportunity to work with him in the evaluation of an alternative fuel. Upon graduation in May of 2010, I hope to attend graduate school and continue to do research on important topics in agriculture.

INTRODUCTION

Ethanol is a renewable energy source that can be created domestically. Derived from plant matter and several grains, most popularly corn in the United States, ethanol is sometimes called grain alcohol (Houghton-Alico, 1982). Ethanol is blended with gasoline and used as a fuel in spark-ignition engines. The two most common blends available for public use are E10 (10% vol (volume) ethanol blended with 90% vol gasoline) and E85 (85% vol ethanol blended with 15% vol gasoline) (Energy, 2007). Ethanol is mixed with gasoline to help boost ethanol's lower heat energy value. Ethanol contains about 29.7 MJ/kg of fuel as opposed to gasoline's heat energy value of around 47.3 MJ/kg of fuel (Engineering, 2007). In theory, an engine would consume about 60% more ethanol than gasoline when fueling the same engine due to ethanol's lower heat value (Lincoln, 1976). However, studies have shown the lower heat values of ethanol are often offset by the fuel's high lubricant qualities, which results in the combustion of only about 15% to 25% more ethanol by volume compared to gasoline (Rothman, 1983).

Small engines produce relatively large amounts of harmful exhaust emissions. In 1991, the United States Environmental Protection Agency (EPA) estimated that small, non-road engines produced 10% of total emissions (Ross, 1999). While newer-generation engines are more efficient and more environmentally friendly, small engines still make a significant contribution to total air pollution loading.

Research has shown that lower compression ratios contribute to the production of emissions from small engines (Al-Baghdadi, 2008). Through manipulation of the compression ratio, Al-Badghdadi was able to combust E85 more efficiently, producing fewer harmful emissions when compared to testing the same engine with the manufacturer-specified compression ratio. Other researchers were able to manipulate the timing of ignition to improve emissions when fueling a small engine on an ethanol blend (Varde, et al., 2007).

The objective of this study was to determine if there were significant ($p < 0.05$) differences in power, torque, specific fuel consumption, and specific exhaust emissions of a small, single-cylinder, spark-ignition engine when fueled with E85 as compared to regular gasoline under two load conditions (governor's maximum and peak torque condition). To reflect how a typical consumer might operate the engine, no modifications were made to the engine with regard to timing or compression ratio.

MATERIALS AND METHODS

Test Fuels. Two 18.9-L (5-gallon) containers of each test fuel were obtained from The Woodshed #3 Convenience Store in Adair, Okla. A sample of each fuel was tested by Magellan Midstream Partners of Kansas City, Kan. (Table 1).

Test Equipment. The power unit for this study was a new Honda GX110 air-cooled, four-stroke, single- cylinder, spark-ignition engine (Table 2). Because a new, in-box

engine was used, we performed the manufacturer's recommended engine break-in procedure prior to the experiment. Engine oil was drained and replaced after break-in was concluded.

The dynamometer used in these tests was a Land and Sea DYNOmite™ water brake absorber (N. H.) with the accompanying DynoMax© software. The power unit and dynamometer were coupled and placed on an engine stand. Dynamometer load was applied to the engine by computer-control using a servo-controlled load valve. This allowed precise and repeatable engine load and speed control.

To determine the size of carburetor jet needed for use with ethanol, we made several torque maps with different sized jets. The jet that resulted in the highest power output was deemed to be the best overall jet for the ethanol fuel. The torque maps for both fuels showed the peak torque engine rpm to be approximately 2800 and the governor's maximum to be approximately 3400 rpm.

Fuel consumption was measured on a mass basis using auxiliary fuel tanks mounted on an Ohaus SD-35™ (Ohaus, Pine Brook, N.J.) digital platform scale (35 × 0.05 kg). A separate but identical fuel tank was used for each fuel in order to avoid cross-contamination. Exhaust emissions were measured with an Auto Logic Gold 5-Gas™ (Auto Logic, Sussex, Wis.) exhaust analyzer. Exhaust manifold temperature was measured with a Raytec AutoPro ST25™ (Raytec, Santa Cruz, Cal.) non-contact infrared thermometer (-32 to 535°C at 1% accuracy) (Fig. 1).

Methods. The order of testing was held in sets of four, 1-h tests as determined randomly. Both fuels were tested under 2 load conditions (governor's maximum and peak torque) with four replications of each level of fuel and load (16 total tests). Before each test, barometric pressure, temperature, relative humidity, and fuel mass were recorded. During the tests, data were manually recorded data every 5 min. Data were collected on fuel mass, power, torque, rpm, exhaust manifold temperature, and specific carbon dioxide (sCO_2), specific carbon monoxide (sCO), specific hydrocarbons (sHC), specific dioxide (sO_2), and specific oxides of nitrogen (sNO_X). The emissions analyzer automatically logged data throughout the duration of the test at 1-s intervals. To switch to a different fuel, the appropriate carburetor jet was installed, the tank was switched and all remaining fuel in the lines and engine was purged.

Test Conditions. All testing was conducted in open-air conditions. To control for differences in ambient conditions, the temperature, barometric pressure, and relative humidity during each test were recorded and used to determine power and torque correction factors (Shelquist, 2009). Subsequent analyses were conducted using corrected power and torque values. Data were analyzed using descriptive statistics and analysis of variance (ANOVA) procedures.

RESULTS AND DISCUSSION

Governor's Maximum Speed. At the 3400 RPM governor's maximum speed, there were no significant differences by fuel for engine torque ($P = 0.37$) or power ($P = 0.41$). There was a significant difference in specific fuel consumption ($P < .0001$) by fuel. When fueled with E85, the engine required 50% more fuel to make almost identical power.

There were no significant differences between fuels in sCO_2 ($P = 0.24$), sCO ($P = 0.22$), sHC ($P = 0.37$), or sNO_X ($P = 0.10$) emissions. Fueling with E85 resulted in significantly lower ($P = 0.03$) sO_2 emissions, with E85 reduced sO_2 emissions by 12.9% compared to regular gasoline (Table 3).

Peak Torque. For peak torque testing (2800 engine RPM), there were significant differences by fuel for engine power ($P = 0.01$) and torque ($P = 0.04$). When compared to regular unleaded gasoline, fueling with E85 decreased engine torque and power by 21.9% and 24.7%, respectively. Fueling with E85 resulted in significantly higher (124%, $P < 0.0001$) specific fuel consumption than did fueling with regular gasoline. There were no significant differences by fuel in sCO_2 ($P = 0.34$), sCO ($P = 0.30$), sHC ($P = 0.053$), sO_2 ($P = 0.88$), or sNO_X ($P = 0.63$) emissions (Table 4).

When fueled with E85, specific fuel consumption was significantly higher when compared to regular unleaded gasoline. This was expected due to ethanol having a lower heat-energy value compared to regular unleaded gasoline. This is somewhat consistent with other research (Al-Baghdadi, Gautam et al.); however, the results shown in this testing indicate far greater fuel consumption by the engine fueled with E85 than other researchers have reported. This may be due to incomplete combustion, especially under peak-torque load, as the carburetor jet was sized to maximize power, not efficiency. Additionally, carburetors have been shown to be less efficient in atomizing ethanol (Al-Baghdadi, 2008) especially at the high flow rates that the engine needs, causing peak torque consumption to trend much higher. Further research is recommended to determine the cause of this finding.

There were no significant differences in torque or power between E85 and regular gasoline at governor's maximum. Although E85 has a lower heat-energy value, the consumption of more E85 offset the energy difference. However, under peak torque conditions, torque decreased by 21.9% and power decreased by 24.7% when fueled with E85 relative to regular gasoline. This difference between regular gasoline and E85 is again inconsistent with what other studies have shown (Al-Baghdadi, Gautam et al.).

After talking with several researchers, the cause of this discrepancy is still not understood. Therefore, more deliberation and study are suggested.

When compared to regular unleaded gasoline, E85 produced no significant reduction in emissions with the exception of decreasing sO_2 emissions by 12.9 per cent under rated speed conditions. It should be noted that all emissions did trend lower when the engine was fueled with E85 but not enough for a significant difference to be found. Other studies (Al-Baghdadi, 2008; Hull, et al., 2006; He, et al., 2003; Varde, et al., 2007; Agarwal, 2007) found a reduction in emissions to some extent, with most reporting significant reductions in CO, CO_2, and NO_X. Though all steps were followed in preparing the emissions analyzer correctly, the data exhibited a large degree of variance. The analyzer may be the root of the discrepancy between the results of this study and others. In future research, a laboratory-grade analyzer should be used instead of the garage-grade analyzer used in this study.

ACKNOWLEDGMENTS

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LITERATURE CITED

Agarwal, A. K. 2007. Biofuels (alcohols and biodiesel) applications as fuels for internal combustion engines. Progress in Energy and Combustion Science. 33, 233-271.

Al-Baghdadi, M. 2008. Measurement and prediction study of the effect of ethanol blending on the performance and pollutants emission of a four-stroke spark ignition engine. Proceedings of the Institution of Mechanical Engineers -- Part D -- Journal of Automobile Engineering. 222: 859-873.

Energy Information Administration. 2007. Energy and economic impacts of implementing both a 25-percent RPS and a 25-percent RFS by 2025. <http://www.eia.doe.gov/oiaf/servicerpt/eeim/notes.html> Accessed Oct. 13, 2007.

The Engineering Tool Box. 2007. Fuels and higher calorific values. <http://www.engineeringtoolbox.com/fuels-higher-calorific-values-d_169.html> Accessed Oct. 12, 2007.

Gautam, M., D. Martin, and D. Carder. 2000. Emissions characteristics of higher alcohol/gasoline blends. Proceedings of the Institution of Mechanical Engineers—Part A—Power & Energy. 214:165-182.

He, B.-Q., J.–X. Wang, J.–M. Hao, X.–G. Yan, and J.–H. Xiao. 2003. A study on emission characteristics of an EFI engine with ethanol blended gasoline fuels. Atmospheric Environment. 37:949-957.

Houghton-Alico, D. 1982. Alcohol fuels: policies, production and potential. Westview Press. Boulder, Colo.

Hull, A., I. Golubkov, B. Kronberg, T. Marandzheva, and J. van Stam. 2006. An alternative fuel for spark ignition engines. International Journal of Engine Research. 7: 203-214.

Lincoln, J. W. 1976. Methanol and other ways around the gas pump. Garden Way Publishing.Vermont.

Ross, D. S. 1999. Clearing the air: OPEI sets the record straighter on 'misleading' news articles. Maryland Cooperative Extension Newsletter. <http://www.bre.umd.edu/agtopics/topics-septoct-99.html> Accessed Oct. 15, 2007.

Rothman, H., R. Greenshields, and F. R. Callé. 1983. Energy from alcohol: the Brazilian experience. University Press of Kentucky. Lexington, Ky.

Shelquist, R. 2009. Engine tuner's calculator. Shelquist Engineering. <http://wahiduddin.net/calc/calc_hp_abs.htm> Accessed October 15, 2007.

Varde, K., A. Jones, A. Knutsen, D. Mertz, and P. Yu. 2007. Exhaust emissions and energy release rates from a controlled spark ignition engine using ethanol blends. The Institution of Mechanical Engineers—Part D—Journal of Automobile Engineering. 221; 933-941.

Table 1. Physical and chemical properties of regular unleaded gasoline and E85.

Fuel property	ASTM test no.	Reg. unleaded	E85
Density (g/ml)	4052	0.7432	0.7798
Heat of combustion (MJ/kg)	240	45.65	35.25
Purity	---------	---------	75.62% vol. ethanol 0.37% vol. methanol

*Note: Analysis by Magellan Midstream Partners, L.P., Kansas City, Kan.

Table 2. Honda GX110 engine specifications.

Bore	57mm
Stroke	42mm
Displacement	107 cc
Rated power	2.61 kW @ 3600 rpm
Rated torque	0.7 kg-m @ 2800 rpm

Table 3. Power, torque, specific fuel consumption, and emissions at governor's maximum (3400 RPM).

	Reg. Unleaded		E85	
Variable	Mean	Std. Dev.	Mean	Std. Dev.
Power (kW)	2.06	0.106	2.11	0.076
Torque (Nm)	5.85	0.347	6.05	0.232
Sfc* (kg/kWh)	0.38	0.018	0.57	0.036
sCO_2 (ppm/kW)	4.24	1.089	3.54	0.096
sCO (ppm/kW)	2.11	0.798	2.73	0.422
sHC (ppm/kW)	289.71	408.799	91.51	11.017
sO_2 (ppm/kW)	2.90	0.266	2.52	0.070
sNOx (ppm/kW)	238.27	180.928	60.19	24.847

*Specific fuel consumption

Table 4. Power, torque, specific fuel consumption, and emissions at peak torque speed (2800 RPM).

	Reg. Unleaded		E85	
Variable	Mean	Std. Dev.	Mean	Std. Dev.
Power (kW)	2.04	0.243	1.54	0.155
Torque (Nm)	6.84	1.024	5.34	0.494
Sfc (kg/kWh)	0.30	0.088	0.67	0.127
sCO_2 (ppm/kW)	3.84	0.575	4.49	0.918
sCO (ppm/kW)	1.69	0.368	2.39	0.979
sHC (ppm/kW)	86.48	16.029	136.88	31.170
sO_2 (ppm/kW)	3.43	0.531	3.56	1.379
sNOx (ppm/kW)	258.61	48.031	189.78	222.977

*Specific fuel consumption

Fig. 1. Experimental setup for fuel testing.

Establishing a rapid and effective method for screening salt tolerance in soybean

Mioko Tamura[*] *and Pengyin Chen*[†]

ABSTRACT

Chlorine (Cl) toxicity has been recognized as a constraint for soybean production. Although the use of a Cl-tolerant crop easily solves the problem, current screening methodologies for Cl tolerance are often ineffective because of inadequate means of detecting and measuring plant response to salinity. In order to facilitate the evaluation process and selection of Cl-tolerant genotypes, a study was conducted to develop a rapid and effective method for screening Cl tolerance in soybean. Seeds of five soybean cultivars, each representing either the includer or excluder genotype to salt stress, were grown in a greenhouse in two different growing media (potting mix or sandy loam) with four different concentrations of sodium chloride (NaCl) solutions. Visual symptoms of Cl toxicity were rated on a 1 to 6 scale (1 as healthy and 6 as dead), and the score was compared with relative shoot/root dry weight and Cl concentration in shoot/root to corroborate the accuracy of the visual ratings. Reduced dry weight was associated with higher Cl concentrations in both root and shoot tissues. The optimal NaCl concentration for screening was determined as 120 mM NaCl since it effectively differentiated excluders from includers. There were negative, significant correlations between relative shoot dry weight and Cl concentration in shoot tissue ($r = -0.91$ $p = 0.05$), and Cl concentration in shoot was also significantly correlated with visual rating score ($r = 0.79$ $p = 0.05$). The presented methodology is simple, rapid, and effective for screening for salt tolerance in soybean.

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[†]Pengyin Chen is an associate professor in the Department of Crop, Soil, and Environmental Sciences.

MEET THE STUDENT- AUTHOR

Mioko Tamura

After my graduation from University of Tsukuba Senior High School in Japan, I came to Fayetteville to study English. I did not know anyone here prior to coming here, so I was very fortunate to meet these people who have given me generous support and made it possible for me to achieve my initial goal, enrolling in the university in fall 2004. I was awarded Harvey A & Jo York, Eddie Davis, and Dale & W Hinkle scholarships to pursue my study. While I was an undergraduate student, I participated some student organizations, such as ICT (International Culture Team) and Organic Farming Club. I began working for Dr. Pengyin Chen during my freshman year, involving a hardness testing of food grade soybean. In 2007, the research project was published in Discovery and also in the Journal of Texture Studies in 2008. In summer 2007, I had an opportunity to be a part of a service learning project on a sustainable school farm in Belize. My career goal is to work for an international corporation in agronomy and rural development. In May 2008, I graduated with a B.S.A. degree in crop management and a minor in agribusiness. The following summer, I started as a graduate assistant in the Department of Crop, Soil, and Environmental Sciences in crop physiology.

I would like to thank Dr. Pengyin Chen for his support and guidance, and I also thank members of the soybean research program and the committee; Drs. Richard Norman, Jennie Popp, and Nathan Slaton for my honors thesis research.

INTRODUCTION

Salt toxicity, as evidenced by high concentration of chlorine (Cl) in soil threatens soybean production worldwide (Essa, 2002). Salt accumulation in the soil profile is mainly caused by poor fertilizer practices or excessive use of irrigation water that results in unbalanced in- and out-flow of groundwater (Lee et al., 2004). The toxicity problem is especially severe in arid and semi-arid areas where higher evaporation rates are expected, but the salt accumulation is also found in many irrigated fields in Arkansas (Wilson et al., 2000). Chloride toxicity problems have arisen in soybean production in the Mississippi River Delta in Arkansas since 1990 (Rupe et al., 2000).

Soybean [*Glycince max*] is one of the main crops in the world for producing edible oil and high-protein livestock feed; however, it is categorized as a "salt sensitive" crop in the stress tolerance subdivisions, exhibiting chlorosis or necrosis on leaves in saline growing conditions (Pantalone et al., 1997). Screening based on the leaf chlorosis score and visual foliar symptoms are considered appropriate for salt-sensitive crops. There are two types of salt response in soybean; includer and excluder. The soybean genotype that translocates Cl to the foliage is called includer whereas excluder stores Cl in the roots. High salt tolerance was associated with Cl exclusion from leaves/shoots (Philip and Broadley, 2001). Thus, yield losses are more severe for includers than for excluders, and Cl causes symptoms ranging from faint foliar chlorosis to plant death as leaf and stem Cl concentrations increased in includers.

Genetic variability of Cl tolerance has a potential use for breeding salt-tolerant soybean. About 20% of soybean cultivars released for the southern U.S. are expected to have an economical salt-tolerance level, yet a practical and economically viable method for screening for Cl tolerance has not been established (Lee et al., 2004). Current screening methodologies for salt-tolerant cultivars are often time consuming and labor intensive; the screening is mainly done by hydroponic culture that requires cautious seedling care, gradual exposure to salinity stress levels, and elaborate nutrient maintenance in the solution. Moreover, the plant tissue analysis is the only measurement used to determine the cultivar's tolerance level to the salt stress.

In order to facilitate the evaluation process and selection of Cl-tolerant genotypes, this research was conducted using soil growing media (commercial soil and sandy loam) to develop a simple and reliable methodology based on foliar symptoms for screening Cl-tolerant soybean genotypes. We hypothesized that a high concentration of NaCl hastens stress symptom development, causes more

severe foliar symptoms, decreases plant biomass of shoots and roots, and increases Cl concentration in shoot and root tissues. Furthermore, includers should have higher Cl concentration in shoot tissue than excluders, whereas excluders should have higher Cl concentration in roots than includers.

The main objective of this study was to develop a rapid and effective methodology for screening Cl tolerance in soybean. The specific objectives were 1) to identify the optimal Cl concentration for screening Cl tolerance based on visual foliar symptoms, and 2) to determine the effects of Cl uptake on root and shoot growth and Cl concentrations in these plant parts.

MATERIALS AND METHODS

Plant Materials and Growth Conditions. Soybean plants were grown in a greenhouse of the Rosen Center at the University of Arkansas, Fayetteville. They were maintained under 14 h daylength and 25°C day / 20°C night temperature throughout the experiment. Seeds of five soybean cultivars ('Clark', 'Williams', 'Dare', 'Lee 68', and 'S-100'; Table 1) were planted in a 9.8-cm square plastic pot, each containing either commercial potting mix (Redi-earth, Vermiculita and Canadian Sphagnum peat moss, Sun Gro Horticulture Distribution Inc., Bellevue, Wash.) or rocky sandy loam soil collected at Kibler, Ark. 'S-100' and 'Lee 68' represented Cl-tolerant cultivars (excluder) and 'Clark', 'Dare', and 'Williams' represented Cl-sensitive cultivars (includer). After the seedlings emerged, only the healthy, uniform-sized plants were maintained from each cultivar for the experiment. Peters nutrient solution (Peter's Plant Food 20-20-20, Spectrum Group, St. Louis, Mo.) was applied once a week after the second trifoliate growth stage.

Three weeks after planting, at the second to third trifoliolate leaf stage, each pot received one of four concentrations of NaCl solution (0, 80, 120, or 160 mM NaCl) to saturate the growing media (100 ml for potting mix and 75 ml for sandy loam). A total of eight combinations (two growing media and four NaCl levels) of treatments were tested on seedlings in five replications (four plants/pot) for each of the five cultivars in the study.

Measurements and Data Analysis. Symptoms were recorded based on the visual rating scale of 1-6 (Fig. 1). The scale was defined as follows; 1 for healthy plant with no chlorosis, 2 for 25% of leaf chlorosis, 3 for 50% of leaf chlorosis, 4 for 75% of leaf chlorosis, 5 for 100% chlorosis, and 6 for complete leaf necrosis and plant death. The application of NaCl solutions were terminated at 13 d after the stress treatment initiation when the difference between includer and excluder cultivars was apparent. Each plant was then rated as the soil appeared to dry out at 4 d after the treatment termination for the potting mix and 7 d for the sandy loam. Average score of the four plants in each pot was used for data analysis. The NaCl concentration that gave the most contrasting differences between sensitive and tolerant cultivars was defined as the critical-selection NaCl concentration for Cl tolerance. Lastly, the samples were oven-dried at 70°C for 7 d. Total shoot dry weights were recorded for the four plants. However, dried roots of all plants from five replications of each treatment were combined. In order to compare genotypic differences effectively, shoot and root dry weights were also converted into relative shoot dry weight (RSDW) and relative root dry weight (RRDW) based on the dry weight of the control [0 mM NaCl] as 1.0. Two out of five replications of bulked dry shoots were randomly chosen and ground for tissue analysis for Cl concentration (CLSH), and bulked dry roots of all five replications were ground for tissue analysis for Cl concentration (CLRT). Samples were deionized water-extracted and analyzed using a spectrophotometer model CIROS ICP (Specttro Analytical Instruments Inc., Mahwah, N.J.).

Data for plant biomass (RSDW and RRDW), Cl concentrations (CLSH and CLRT), and visual rating score (RATE) were subjected to analysis of variance. Honest significant difference (Tukey) was used to compare means between the includer and excluder cultivars and NaCl concentrations ($P = 0.05$) in each growing media (potting mix or sandy loam), separately. Simple correlation was used to assess relationships among RSDW, RRDW, CLSH, CLRT, and RATE. All statistical analyses were done by the SAS version 9.2 (SAS Institute, Cary, N.C.).

RESULTS AND DISCUSSION

Symptoms of Chloride Toxicity in Shoot and Roots. Visible symptoms, as reflected by visual rating score, were observed on plants in all Cl concentrations in both potting mix and sandy loam soil (Table 2). Symptoms of Cl toxicity ranged from burning of leaves and stunting to interveninal chlorosis and premature chlorosis. Symptom severity increased with NaCl concentration in both growing media. Symptoms were more severe in potting mix than in sandy loam in general. For instance, only a few excluder plants showed chlorosis symptoms with 80 mM NaCl in sandy loam while all plants in potting mix showed 25% or more chlorosis with the same NaCl concentraion. However, visual rating scores of includers were consistently higher than those of excluders at all levels of Cl in potting mix or sandy loam growing media. Statistical analysis showed that the significant difference in visual symptom score between includers and excluders was observed in 120 mM with potting mix (Table 2, illustration and Fig. 2). However, none of the NaCl concentrations with sandy loam showed statistically significant differences although 120

mM NaCl concentraton showed trends toward increased ratings in includers regard to excluders.

Valencia et al. (2008) used a hydroponic culture system to identify a threshold NaCl concentration for Cl toxicity in soybean and found that 120 mM NaCl was the critical NaCl concentration for tolerance selection, which separated includers from excluders at 14 days of Cl stress treatment when interveninal chlorosis on leaves was observed among includers while excluders remained healthy. This agrees with our results using potting mix.

Symptoms of Cl toxicity were also evident in the root systems (Fig. 3). Under the highest concentration of NaCl (160 mM), both includers and excluders developed thin and dark roots with extremely short and sparse secondary roots. Root development was greatly reduced even at the 80 mM NaCl compared to the control. However, root system damage from NaCl cannot be feasibly used as a selection criterion for salt tolerance due to the extensive work involved in root damage evaluation.

Effects of Salt on Plant Biomass. For RSDW and RRDW, relative weights decreased numerically, but not always significantly, as the applied NaCl concentration increased; the higher NaCl concentrations caused more sever toxicity and reduced biomass. A similar inverse relationship between NaCl concentration and plant biomass was also reported by Valencia et al. (2008) using commercial cultivars and by Kao et al. (2006) using wild soybeans.

The RSDW of includers were always, but not significantly, lower than those of excluders. Includers translocate Cl to shoot tissues; therefore, they had more interference with growth due to applied NaCl solution than excluders. In contrast, the RRDW of excluders were generally lower than those of includers though none of the differences were significant. Excluders store Cl in roots, thus the root growth of excluders were hindered by the NaCl solutions by a greater extent than includers.

Overall, RRDW had lower values than RSDW, which indicates that roots were more affected by salinity than shoots. This was also observed by Valencia et al. (2008) and Kao et al. (2006). However, our results conflict with Essa's (2002) finding that root dry weight was less sensitive to salt stress than shoot dry weight (2002).

In comparing the growing media, we found that the sandy loam had higher values for both RSDW and RRDW than the potting mix, suggesting plants grown in sandy loam were less affected by salt stress than those in potting mix. There was twice as much difference between the overall average RRDW of potting mix (0.24) and sandy loam (0.51). In contrast, the average RSDW between potting mix and sandy loam were very similar (0.50 and 0.56, respectively). This result agreed with our observations of visual symptoms that potting mix showed more severe symptoms than sandy loam at the same NaCl concentraions.

Chloride Concentrations in Shoots and Roots. Numerically higher Cl content in shoots (CLSH) was always found in includers than in excluders (Table 5). In contrast, numerically higher Cl content in roots (CLRT) was found in excluders than in includes (Table 6). There were very few significant differences in the means; however, these results demonstrated that the excessive Cl was translocated into leaves in includer cultivars, but it was restricted and accumulated in the root system in excluder genotypes.

Chloride concentrations in both shoots and roots had positive linear relationships with NaCl concentrations (Table 7), which agrees with the report by Essa (2002). This showed that increased, applied NaCl concentration resulted in increased tissue Cl concentrations.

The Cl concentration reached a plateau at 120 mM NaCl concentration for the potting mix. However, CLSH of sandy loam exhibited a linear increase with Cl concentrations up to 160 mM NaCl. The significant difference between includers and excluders was shown at 80 mM NaCl concentration with sandy loam. Overall, shoot from plants grown in potting mix had higher Cl concentration than those in sandy loam.

The significant differences in CLRT were only seen between control and 80 mM NaCl concentration or above in both potting mix and sandy loam, whether for includes and excluder plants. This implied that roots reached their nearly maximum Cl intake capacity with 80 mM NaCl solution. Unlike CLSH, roots of plants grown in sandy loam contained more Cl than those grown on in potting mix, probably because plants grown in sandy loam had higher RRDW than those in potting mix.

Correlations Among Measurements. There were positive, significant correlations between RSDW and RRDW (Table 7). Positive and significant correlations were also found between CLSH and both CLRT and RATE. The negative, significant correlations between CLSH/CLRT and biomass (RSDW and RRDW) imply that higher Cl concentration in shoots/roots limited plant growth to a greater extent. Finally, the validity of visual rating scale (RATE) was supported by the significant correlation between RATE and both RSDW (negative) and CLSH (positive). In other words, a higher degree of visual symptoms was a sign of reduced shoot growth and increased Cl concentration in shoot. This result agrees with reports by Pantalone et al. (1997) and Lee et al. (2004).

The trends of negative or positive correlation among measurements were found to be exactly the same between potting mix and sandy loam; however, the stronger correlations were found in sandy loam than in the potting mix between CLRT and both RSDW and RRDW. Although the correlation between RATE and RSDW was not significant for potting mix, this correlation was significant for the sandy loam.

Our research showed that the visual, foliar evaluation of soybean for chloride tolerance screening with 120 mM NaCl is a rapid and effective screening method with both potting mix and sandy-loam media. Additional experiments are ongoing to confirm these results.

ACKNOWLEDGMENTS

Financial support for this project was provided by an Honors College Undergraduate Research Grant, the Arkansas Soybean Promotion Board, and the University of Arkansas Agricultural Experiment Station. The author wishes to thank Dr. Pengyin Chen, honors thesis mentor; Drs. Richard Norman and Jennie Popp, thesis committee members; Dr. Nathan Slaton for plant tissue analysis; and Dr. Tetsuaki Ishibashi for statistical analyses. All of their help was necessary for the success of the project and is greatly appreciated.

LITERATURE CITED

Essa, T.A. 2002. Effect of salinity stress on growth and nutrient composition of three soybean (*Glycine max* L. Merrill) cultivars. J. Agron.Crop Sci.188:86-93.

Kao, W., T.T. Tyng, C.T. Hung, and N.S. Chen. 2006. Response of three Glycine species to salt stress. Environmental and Experimental Botany. 56:120-125.

Lee, G.J., H.R. Boerma, M.R. Villagarcia, X. Zhou, T.E. Carter Jr., Z. Li, and M.O. Gibbs. 2004. A major QTL conditioning salt tolerance in S-100 soybean. Theor. Appl. Genet. 109 (8):1610-1619.

Pantalone, V.R., W.J. Kenworthy, L.H. Slaughter, and B.R. James. 1997. Chloride tolerance in soybean and perennial Glycine accessions. Euphytica 97(2):235-239.

Philip, J. and R. Broadley. 2001. Chloride in soils and its uptake and movement with the plant: A review. Annals of Botany. 88(6):967-988.

Rupe, J.C., J.D. Widick, W.E. Sabe, R.T. Robbins, and C.B. Becton. 2000. Effect of chloride and soybean cultivar on yield and the development of sudden death syndrome, soybean cyst nematode, and southern blight. Plant Dis. 84:669-674.

Wilson, C.E. Jr., T.C. Keisling, D.M. Miller, C.R. Dillon, A.D. Pearce, D.L. Frizzell, and P.A. Counce. 2000. Tillage influence on soluble salt movement in silt loam soils cropped to paddy rice. Soil Sci. Soc. Am. J. 64:1771-1776.

Valencia, R., P. Chen, T. Ishibashi, and M. Conatser. 2008. A rapid and effective method for screening salt tolerance in soybean. Crop Sci. 48:1173-1779.

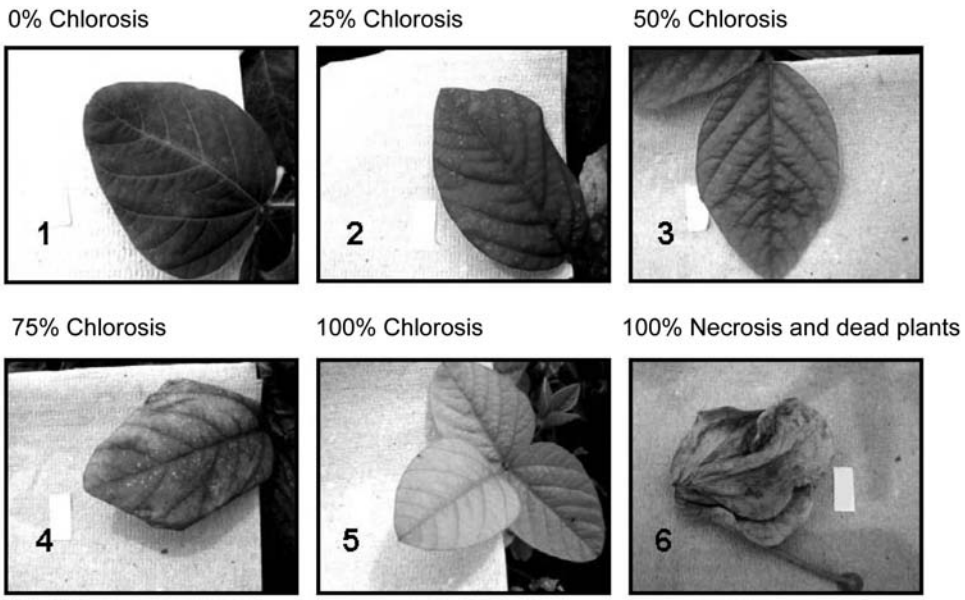

Fig. 1. Visual rating scale (1~6) used for evaluating foliar chloride symptoms in soybean.

Fig. 2. Comparison of the symptoms of at 13 d on 'Clark' (left, includer) and 'Dare' (right, excluder) soybean in potting mix with 120 mM NaCl.

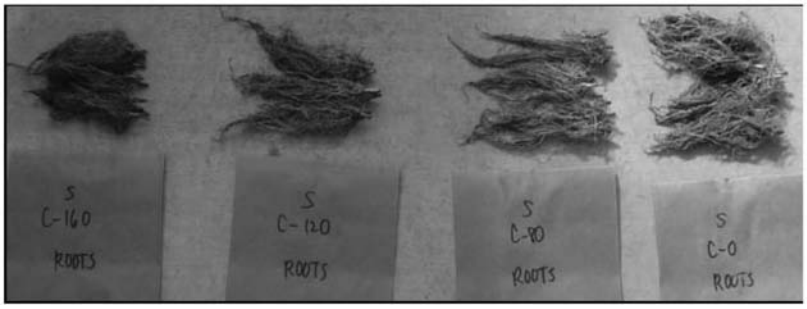

Fig. 3. Root development of Clark soybean (includer) in sandy loam soil with 160, 120, 80, and 0 mM NaCl, respectively.

Table 1. Soybean cultivar with known reactions to salt used in this greenhouse study to evaluate the response to salt stress.

Genotypes	Reaction	Classification
Clark	Sensitive	Cl-includer
Dare	Sensitive	Cl-includer
Williams	Sensitive	Cl-includer
Lee 68	Tolerant	Cl-excluder
S-100	Tolerant	Cl-excluder

Table 2. Visual rating scores of foliar chlorosis symptoms averaged for five soybean cultivars grown in potting mix and sandy loam with different concentrations of NaCl.

NaCl concentration	RATE†							
	Potting Mix				Sandy loam			
(mM)	Includer		Excluder		Includer		Excluder	
80	3.56	Aa‡	2.33	Ba	2.86	Ba	1.87	Aa
120	4.57	Aa	3.29	ABb	4.55	Aa	3.28	Aa
160	5.25	Aa	3.88	Aa	4.92	Aa	3.74	Aa
Mean	4.46		3.17		4.11		2.96	

† RATE = Foliar symptoms rating scale; 1 for healthy plant with no chlorosis, 2 for 25% of leaf chlorosis, 3 for 50% of leaf chlorosis, 4 for 75% of leaf chlorosis /scorching, 5 for 100 % chlorosis, and 6 for completely dead plant.

‡ In a column, scores followed by the same upper case letter are not significantly different by Tukey (p=0.05).
Scores of includer and excluder in a row followed by the same lower case letter are not significantly different by Tukey (p=0.05).

Table 3. Relative shoot dry weights (RSDW) of five soybean cultivars grown in potting mix and sandy loam with different concentraions of NaCl.

NaCl concentration	RSDW (g)†							
	Potting mix				Sandy loam			
(mM)	Includer		Excluder		Includer		Excluder	
0	1.00	A‡	1.00	A	1.00	A	1.0	A
80	0.59	Ba	0.68	Aa	0.60	Ba	0.63	Ba
120	0.43	BCa	0.44	Aa	0.50	Ba	0.66	Ba
160	0.37	Ca	0.49	Aa	0.44	Ba	0.54	Ba
Mean	0.46		0.54		0.51		0.61	

† Relative Shoot Dry Weight = treated / control; averaged over genotypes.

‡ In a column, relative dry weight followed by the same upper-case letter are not significantly different by Tukey (p=0.05).

The relative dry weights of includer and excluder in a row followed by the same lower-case letters are not significantly different by Tukey (p=0.05).

Table 4. Relative root dry weights (RRDW) averaged over five soybean cultivars grown in potting mix and sandy loam with different concentrations of NaCl.

NaCl concentration	RRDW (g)†							
	Potting mix				Sandy loam			
(mM)	Includer		Excluder		Includer		Excluder	
0	1.00	A‡	1.00	A	1.00	A	1.0	A
80	0.43	Ba	0.38	Aa	0.66	ABa	0.58	ABa
120	0.12	Ba	0.17	Aa	0.51	Ba	0.47	Ba
160	0.21	Ba	0.14	Aa	0.49	Ba	0.37	Ba

† Relative root dry weight = treated / control; averaged over genotypes.

‡ In a column, relative dry weights followed by the same upper case letter are not significantly different by Tukey (p=0.05).

The relative dry weights of includer and excluder in a row followed by the same lower-case letter are not significantly different by Tukey (p=0.05).

Table 5. Chloride concentrations in shoot (CLSH) averaged for five soybean cultivars grown in potting mix and sandy loam with different concentrations of NaCl.

NaCl concentration	CLSH† (mg/Kg)							
	Potting Mix				Sandy Loam			
(mM)	Includer		Excluder		Includer		Excluder	
0	5229	Ca‡	1552	Cb	3404	Ca	2512	Ca
80	46583	Ba	40467	Ba	41463	Ba	24743	Bb
120	61478	Aa	50689	Aa	46788	Ba	34017	Ba
160	60768	Aa	49632	Ab	64640	Aa	54769	Aa
Mean	43515		35585		39074		29010	

† CLSH= Chloride concentration in shoots (mg/Kg).
Plants were grown in either potting mix or sandy loam with 0, 80, 120, or 160 mM NaCl. Cl concentrations were determined by tissue analysis.

‡ In a column, chloride concentrations followed by the same upper case letter are not significantly different by Tukey (p=0.05).

The chloride concentrations of includer and excluder in a row followed by the same lower-case letter are not significantly different by Tukey (p=0.05).

Table 6. Chloride concentrations in root (CLRT) averaged for five soybean cultivars grown in potting mix and sandloam with different concentrations of NaCl.

NaCl concentration	CLRT† (mg/Kg)							
	Potting mix				Sandy loam			
(mM)	Includer		Excluder		Includer		Excluder	
0	7523	Ba‡	1984	Ba	18920	Ba	21765	Ba
80	19717	Aa	26398	Aa	36933	Ab	42225	Aa
120	18487	Ab	32775	Aa	42133	Aa	48900	Aa
160	18420	Aa	40294	Aa	36350	Ab	47625	Aa
Mean	16037		25363		33584		40129	

† CLRT= Chloride concentration in roots (mg/Kg).
Plants were grown in either potting mix or sandy loam with 0, 80, 120, or 160 mM NaCl.
Cl concentrations were determined by tissue analysis.
The chloride concentrations of includer and excluder in a row followed by the same lower-case letter are not significantly different by Tukey (p=0.05).

‡ In a column, chloride concentrations followed by the same upper-case letter are not significantly different by Tukey (p=0.05).

Table 7. Correlations among relative shoot, root dry weights, shoot and root chloride concentrations, and rating scores of Cl toxicity of NaCl stressed soybean.

Overall	RSDW	RRDW	CLSH	CLRT	RATE
RSDW		0.81***	−0.91***	−0.48**	−0.52**
RRDW			−0.83***	−0.40*	−0.30NS
CLSH				0.44**	0.79***
CLRT					−0.27NS
RATE					

Potting Mix	RSDW	RRDW	CLSH	CLRT	RATE
RSDW		0.91***	−0.93***	−0.61**	−0.48NS
RRDW			−0.91***	−0.72***	−0.22NS
CLSH				0.59**	0.84***
CLRT					−0.34NS
RATE					

Sandy Loam	RSDW	RRDW	CLSH	CLRT	RATE
RSDW		0.69***	−0.88***	−0.74***	−0.56*
RRDW			−0.77***	−0.83***	−0.34NS
CLSH				0.69***	0.80***
CLRT					−0.15NS
RATE					

*Significant at P < 0.05.
**Significant at P < 0.01.
***Significant at P <0.001.
RSDW= Relative shoot dry weight.
RRDW= Relative root dry weight.
CLSH= Chloride concentration in shoot.
CLRT= Chloride concentration in root.
RATE= Rating score of visual Cl toxicity symptoms.
NS= Not significant.

Instructions for Authors

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PARTS OF THE MANUSCRIPT

The title page should include the following:

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The *Abstract* summarizes the purpose, procedures, and main findings in 250 words or less.

The *Introduction* states the purpose of the study, the hypothesis, and pertinent background information.

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