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Several Authors

DISCOVERY

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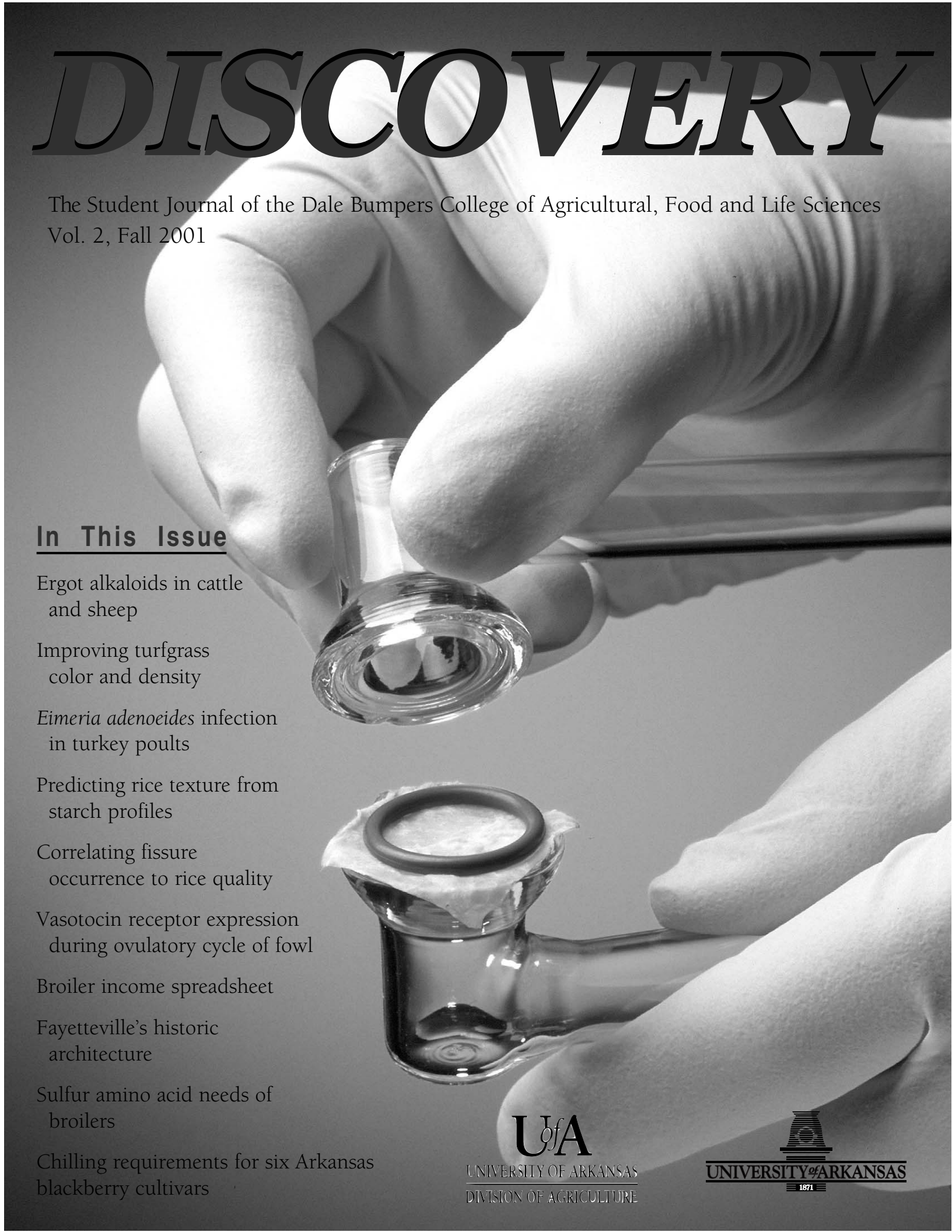

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The Student Journal of the Dale Bumpers College of Agricultural, Food and Life Sciences

Vol. 2, Fall 2001

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Call for Papers

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Undergraduate Research Articles

Cover photo by Scott Bauer (glass parabiotic chamber used to test animal stomach tissue for permeability in the presence of alkaloids)

Letter from the Dean

Gregory J. Weidemann

Our second edition of the *Discovery* journal could just as well be named *Opportunity*. It represents the abundant opportunity in the Bumpers College of Agricultural, Food and Life Sciences for undergraduate students to work with faculty mentors and graduate students on research and creative projects.

Discovery provides the opportunity for our scholars to publish the results of their work. In the academic world, publication is stressed as the culmination of scholarly activity that contributes to a body of knowledge. By providing details of how a project is conducted and a careful analysis of results, the author invites other researchers to examine the conclusions and see if they fit as pieces of the puzzle on which they are working.

Discovery provides a showcase for the accomplishments of these student authors and for the dedication of their mentors. Congratulations authors! Thank you mentors! We are proud of you.

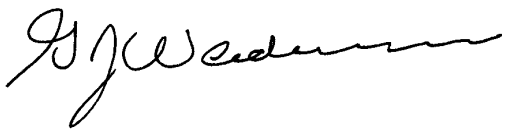
Discovery illustrates another point that we can't make too often—the synergy created by the linkage of Division of Agriculture research and extension programs with the academic programs of the College. These articles are prime examples of how the education our students receive is impacted by the work of their professors as Arkansas Agricultural Experiment Station scientists.

Many of the student authors are enrolled in the Bumpers College Honors Program, which encourages scholarly work beyond the normal course work. Simply put, Honors Program students can get more from their education by putting more into it.

This second issue of *Discovery* includes an article from a study of the architectural heritage of Fayetteville. Scholarly work considered for publication is not restricted to projects normally associated with agriculture or laboratory-based research. We invite manuscripts on social science and creative projects from disciplines that make up the School of Human Environmental Sciences, which is a major part of the Bumpers College.

Another article in this issue is by first- and second-year undergraduate students who completed an innovative course in which the faculty teach methods they are using for their own scientific research. The students conducted experiments that provide insight into the immune response of turkey poults to a protozoan parasite.

Discovery is a clear example of our commitment in the Bumpers College of Agricultural, Food and Life Sciences to the University of Arkansas vision of a nationally competitive, student-centered research university serving Arkansas and the world.



Gregory J. Weidemann, Interim Dean and
Associate Director, Arkansas Agricultural
Experiment Station

The possible enzymatic differences between cattle and sheep in their response to ergot alkaloids

Susan M. Cannon,[*] *Charles F. Rosenkrans, Jr.,*[§] *and Ali Moubarak* [¶]

ABSTRACT

Ergotamine is an ergot alkaloid associated with fescue toxicosis of livestock who have grazed endophyte-infected fescue. High performance liquid chromatography (HPLC) was used to detect individual or species-specific differences in the metabolism of ergotamine by liver cytochrome P450 of sheep and cattle. Livers were collected from four steers and two sheep. The diet of the steers used in this study consisted of two being fed a grain diet, one steer grazing endophyte-infected fescue, and the final steer grazing endophyte-free fescue. The two lambs were both fed a grain diet. Livers were prepared and examined for the disappearance of ergotamine and its isomer by HPLC analysis. Liver microsomes from cattle appeared to metabolize ergotamine to a greater degree than those from sheep. There were no apparent differences in the metabolism of ergotamine when comparing cattle that grazed endophyte-infected fescue to cattle that grazed endophyte-free fescue. Therefore, diet had no effect on the metabolism rate of ergotamine. This work provides insight into the possible genetic differences between species-specific and individual animals. Further study of such differences should improve breeding programs and produce animals that can more effectively tolerate fescue toxins.

[*] Susan M. Cannon is a senior majoring in agricultural education with a minor in communication.

[§] Charles F. Rosenkrans, Jr., faculty sponsor, is a professor in the Department of Animal Science.

[¶] Ali Moubarak, faculty sponsor, is a research associate in the Department of Animal Science.

INTRODUCTION

Cattle in the Midwest and South routinely graze pastures consisting of tall fescue (*Festuca arundinacea*) infected with the endophytic fungus *Neotyphodium coenophialum*. The fungus secretes ergot alkaloids that enhance stress-tolerance of the plant but result in poor performance by some of the cattle grazing the infected herbage. It is estimated that tall fescue is grown on over 14 million ha in the United States (Steudemann and Hoveland, 1988). Although fungal endophytes of grasses have been known since the 1930s, their economic importance was not recognized until an association was made in the late 1970s between *Neotyphodium coenophialum* and a toxicity syndrome in livestock consuming tall fescue (Hoveland, 1993). This syndrome is known as fescue toxicosis and is often referred to as 'summer slump' or 'summer syndrome' due to the unthrifty animal appearance and poor performance during summer (Schmidt and Osborn, 1993). This widespread syndrome is characterized by poor animal gains, intolerance to heat, excessive salivation, rough hair coat, elevated body temperature, poor appetite, nervousness, lower milk production, and reduced conception rate (Hoveland, 1993). Additionally, animal behavior is altered in that animals seek shade, stand in water, and consequently spend less time grazing during the hot part of the day (Bond et al., 1984; Steudemann et al., 1986).

Trials have been performed to determine how much performance is lost in relation to fescue toxicosis. A study on milk production found that performance was reduced by as much as 45% in beef cows (Schmidt, et al., 1983), 50% in beef heifers, and 60% in dairy cows (Hemken, et al., 1979). A study of the pregnancy rate of heifers on endophyte free (E-), as compared with infected (E+) tall fescue pasture, concluded that pregnancy was reduced from 96% to 55% in those who grazed endophyte infected (E+) pasture (Schmidt, 1986). A third study related to calf weaning weights, concluded that weaning weights were reduced by 23 kg/calf because of fescue toxicosis (Hoveland, 1993). Together, losses from reduced conception rates and weaning weights are estimated at $600 million annu-

Meet the Student-Author

I am a senior from Ashdown majoring in agricultural education, with a minor in communication. I plan to graduate in December of 2001. As a student at the University of Arkansas I have received several scholarships and have had the opportunity to participate in many extracurricular and scholastic activities. I am a member of the Collegiate Livestock Judging Team, Agricultural Communicators of Tomorrow, Associate Student Government, University Programs, Alpha Zeta Fraternity, and the Gamma Sigma Delta Agricultural Honorary Society. Additionally, I am currently serving as a Dale Bumpers College of Agricultural, Food and Life Sciences Ambassador.

I chose this research project because of the significant effects of fescue toxicosis on cattle and sheep. This experience has allowed me to enhance my research techniques as well as write a paper for publication. These skills will not only be beneficial to me as I further my education but also as I advance into my professional career.

I would like to express my thanks to Dr. Ali Moubarak and Dr. Charles F. Rosenkrans for their support and guidance throughout this research project.

Susan Cannon

ally in the United States (Paterson, et al., 1995).

Unlike beef, the characteristics of fescue toxicosis exhibited in sheep that have grazed endophyte-infected fescue are relatively small. Studies have shown that ewes grazing endophyte-infected fescue have decreased prolactin and lengthened intervals from introduction of the ram until conception. However, trials evaluating sheep that have grazed endophyte-free (less than 1% infected) and endophyte-infected fescue (greater than 95% infected) have shown that mean daily respiration rates and heart rates, rectal temperature, and hematocrit were not affected by the endophyte-infected fescue (Fiorito, et al., 1991). In addition, a study performed by Rankins (1996) showed that 15 crossbred sheep with a diet of endophyte-free fescue (0% infected, 50% hay, 40% seed, 10% molasses) and endophyte-infected fescue (95% infected, 50% hay, 40% seed, 10% molasses) exhibited no differences. This study concluded that sheep fed the endophyte-infected fescue did not portray typical fescue toxicosis (Rankins, 1996).

Several methods of pasture management have been researched to ameliorate the effects of fescue toxicosis. These methods include interseeding with clovers, preventing the formation of seed heads either by overstocking or clipping, pasture renovation, moving cattle to non-fescue pasture during hot weather, use of plant growth regulators, supplementing with grain or 50:50 broiler litter/shelled corn mix, and use of creep feed or creep grazing with cow-calf pairs (Schmidt and Osborn, 1993). Studies have shown that interseeding endophyte-infected fescue pastures with clover improved pregnancy rates of cows, improved gains of grazing steers, and increased calf weaning weights. However, the pregnancy rates were not improved to levels considered economical for the beef industry (Schmidt and Osborn, 1993). In addition, calves continuously grazing endophyte-infected fescue at lower stocking rates appeared to have more severe toxicosis characteristics because of the tendency to selectively graze seed heads, even though the endophtye is concentrated there (Schmidt and Osborn, 1993).

Many possible methods have been proposed to reduce fescue toxicosis; however, none of the proposed solutions have alleviated the syndrome, and fescue remains a widely used forage crop due to its wide range of adaptation, ease of establishment, tolerance of poor soil and climatic conditions, and long grazing season with good winter growth.

In 2000, Moubarak and Rosenkrans reported that the liver enzyme, cytochrome P450 3A, was present and has been shown to metabolize fescue toxins in cattle. Cytochrome P450 enzymes constitute a superfamily of heme-thiolate proteins that catalyze the primary oxidation of a wide variety of natural endogenous substrates like steroids, fatty acids, prostaglandins, leukotrienes, and lipid hydroperoxides. They also play an important role in the metabolism of exogenous compounds like drugs, procarcinogens, solvents, anesthetics, and environmental pollutants (Peyronneau, et al., 1994).

Our objective was to determine if HPLC analysis could be used to detect individual or species-specific differences in the metabolism of ergotamine by liver microsomes of sheep and cattle. Ergotamine is one of many ergot-alkaloids and is our test compound.

MATERIALS AND METHODS

Livers were obtained from four steers and two lambs. The animals were taken from experiments that had been approved by the University of Arkansas' Institutional Animal Care and Use Committee. The nutritional diet of the four steers (450 to 650 kg body weight) used in this study consisted of two steers being fed a grain diet, one steer grazing endophyte-infected fescue, and the final steer grazing endophyte-free fescue. The two lambs (80 to 100 kg body weight) used in this study were both fed a grain diet.

Liver microsomes were prepared according to Kremers, et al. (1981). Liver tissues (50 to 100 g) were collected and frozen in Collins buffer. Frozen samples were thawed, washed in sodium chloride (150 mM), and homogenized in a sucrose Tris-buffered medium. Microsomes were prepared according to a procedure consisting of a three-step centrifugation process of the tissue homogenate. The first centrifugation was at 800 xg for 10 minutes. The supernatant was collected and the second centrifugation was performed at 13,500 xg for 20 minutes. Centrifugation was done a third time at 105,600 xg for 60 minutes. Supernatant from the final centrifugation procedure was discarded, the pellet resuspended in a sodium phosphate/glycerol solution, and protein content determined. Microsome suspensions were stored in a freezer at -20°C until used within 20 to 30 days.

Ergotamine reactions were prepared according to Peyronneau, et al. (1994) and Moubarak and

Rosenkrans (2000). Microsomes (50 µl of protein) were incubated in microcentrifuge tubes containing ergotamine (20 µl) and a NADPH generating system for 30 minutes at 37°C. Immediately following the incubation, the enzymatic reaction was stopped by adding a deproteinizing agent (94% acetonitrile, 6% glacial acetic acid). Reaction tubes were centrifuged at 12,000 xg for 4 minutes and the supernatant was collected. Tubes had a total volume of 500 ml. Twenty µl of each supernatant from the enzyme assays were examined for the disappearance of ergotamine and its isomer by HPLC analysis (Moubarak, et al., 1993).

RESULTS AND DISCUSSION

Metabolites M-1, M-2, and M-3, as well as M1-Iso and M2-Iso are exhibited in cattle; however, there were no signs of these metabolites displayed in sheep (Fig. 1). Ergotamine metabolism in cattle and the appearance of metabolites (M-1, M-2, M-3) in Fig. 2 show that ergotamine is gradually reduced by 50% during the first 30 minutes of incubation, while metabolites are increased as a function of incubation time. The time course for ergotamine metabolism by sheep and the unvarying rate of metabolites indicate that ergotamine is gradually reduced by only 10% during the first 30 minutes of incubation, while metabolites remain constant at 0% (Fig. 3).

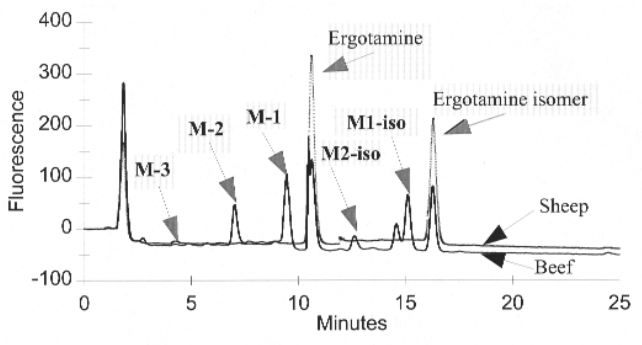


Fig. 1. HPLC chromatogram of products from ergotamine incubation with liver microsomes from cattle and sheep grazing endophyte-infected tall fescue.

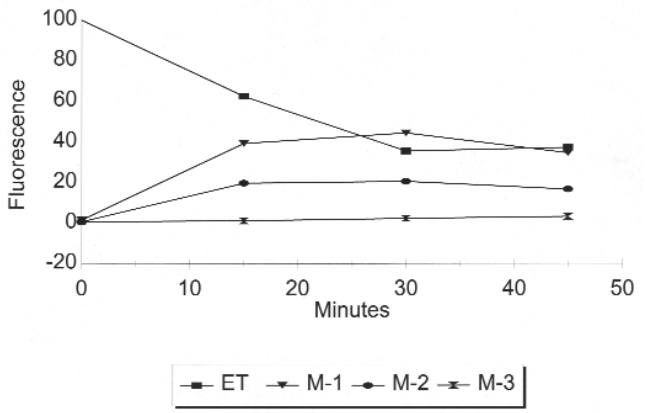


Fig. 2. Time dependent formation of metabolites M-1, M-2, and M-3 when ergotamine was incubated with liver microsomes from cattle.

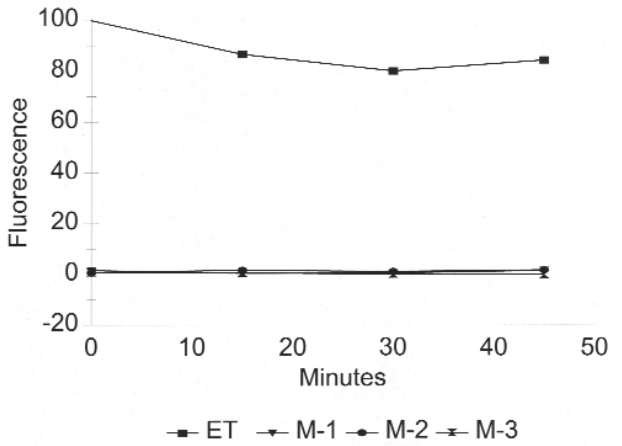


Fig. 3. Time dependent formation of metabolites M-1, M-2, and M-3 when ergotamine was incubated with liver microsomes from sheep.

Based on these data, cattle liver microsomes appear to metabolize ergotamine differently than do those of sheep. On the other hand, there were no apparent differences in the metabolism of ergotamine when comparing cattle that grazed endophyte-infected fescue to cattle that grazed endophyte-free fescue. However, the lack of differences could be due to the small number of animals studied in this experiment. This experiment was exploratory in nature and further work to investigate such differences is in progress.

This work provides insight into the possible genetic differences between species and individual animals that were fed dissimilar diets. When the genetic marker for animals with a high tolerance for fescue toxins is identified, such information will be useful in selecting animals for breeding programs to produce livestock that can more effectively tolerate high levels of fescue toxins.

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Improvements in turfgrass color and density resulting from comprehensive soil diagnostics

Matt Cordell,[*] *Jonathan Davis,*[§] *and David E. Longer*[¶]

ABSTRACT

There are roughly 220 golf courses in Arkansas, and as many as 50% of these courses were constructed using common bermudagrass fairways. Although resilient, common bermudagrass loses density and quality over time. In this experiment physical and chemical properties of the soil were analyzed to determine the causes of decline in turf quality observed on several fairways of a local golf course. Once a particular fairway was selected for study and preliminary soil sampling conducted, GS+, a geostatistical computer program, was used to map the location of certain chemical deficiencies. A moderate to severe Mg deficiency was detected throughout the fairway. Twelve different fertility treatments were designed to enhance the overall density, texture, and color of the turf. Magnesium sulfate ($MgSO_4$), Primo™ (a plant growth regulator), and Nitron (an organic nitrogen source) all showed significant improvements in turf quality. Extensive and comprehensive soil testing was found to be very beneficial; "hidden" nutrient deficiencies were discovered, which allowed site-specific treatments to be included in the test.

* Matt Cordell is a senior majoring in crop management in the Department of Crop, Soil and Environmental Sciences.

§ Jonathan Davis was a graduate assistant in the Department of Crop, Soil, and Environmental Sciences.

¶ David E. Longer, faculty sponsor, is an associate professor in the Department of Crop, Soil, and Environmental Sciences.

INTRODUCTION

Common bermudagrass, (*Cynodon dactylon*), is a hardy grass species that is widely used for sports fields and lawns in the southern United States. Many golf course fairways in Arkansas consist partly or entirely of common bermudagrass. Advancements have been made to increase vitality and appearance of grasses, but problems can arise. Common bermudagrass, though hardy, can experience problems with color, texture, and density, which may reduce its desirability as a fairway grass (D.E. Longer, personal communication). A number of factors may lead to poor stands. Disease, pest damage, mineral deficiencies, water stress, and physical properties of soil can all affect the overall appearance of a bermudagrass fairway. Golf courses are usually monitored closely for disease and pests; therefore, any noticeably reduced turf quality might result from chemical and physical soil properties.

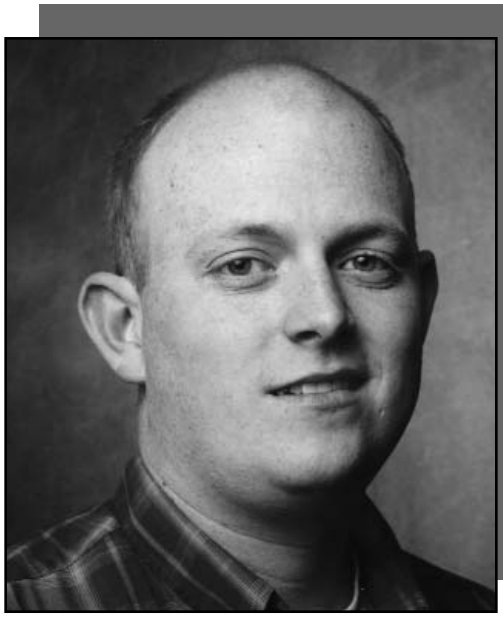
Numerous nutrient amendments are available and can be added to a soil to increase the color, texture, and density of turf. However, in some cases standard nutrient management programs are not always effective in improving grass quality. The lack of micronutrients can be the cause for poor quality stands (Hummell, 1996). Also, soil physical properties can significantly affect the availability of applied nutrients to the plants. Bulk density and soil texture are the two main physical properties that may alter nutrient availability (Turner, 1992).

Bulk density is soil mass per unit of volume and is usually expressed in kg/m^3. If the soil has a high bulk density, plant roots may not be able to reach water and nutrients that exist deeper in the soil. Root growth and penetration is often inhibited in soils with a bulk density equal to or greater than 1600 kg m^{-3} (Brady and Weil, 1999). Increased bulk density also impedes water infiltration and may cause ponding of water on the surface, which can lead to water logging in the roots, cre-

Meet the Student-Author

I am from Hampton and graduated from Hampton High School in 1998. I am a senior majoring in crop management in the Department of Crop, Soil and Environmental Sciences. I have received the Romeo E. Short, Joseph E. Fleming, Arkansas Plant Food Association, Staple Cotton, and Fontaine R. Earle Crop Science scholarships. I have been involved in many activities while attending the University of Arkansas, including being a New Student Orientation Leader, an active member and officer in Collegiate 4-H/FFA, an executive officer in FarmHouse Fraternity for three years, and serving as a Dale Bumpers College of Agricultural, Food and Life Sciences Student Ambassador. This past summer I was honored to be a part of the Adair/Bollenbacher internship program in the Department of Plant Pathology here at the University.

I decided to do this project upon the encouragement of my advisor, Dr. David Longer. I learned many things about turf grass, soil testing, soil fertility, and general factors that affect plant growth. After completing my bachelor's degree, I plan to continue on with graduate school and further my education in some agronomic field. I learned a lot about the research process, and I feel better prepared to enter a graduate degree program. All in all, I consider this a great experience that will assist me in the future.



Matt Cordell

ating an anaerobic environment that is not conducive to nutrient uptake or plant growth (Brady and Weil, 1999).

Soil texture refers to the relative amounts of sand, silt, and clay in a soil. The texture of the soil may also play a vital role in turf vigor and growth. In finer textured soils, such as clay loams, there is an increase in water holding capacity and cation exchange capacity. In coarse textured soils, such as sandy loams, there is increased infiltration of water and leaching of surface applied nutrients. This increased water infiltration in sandy soils may cause leaching of essential nutrients that are not commonly applied.

Turf texture and density are commonly rated on a scale from 1 to 9, 9 being the most desirable. Initial observations were taken in June 2000; the fairway showed poor leaf color, reduced turf density, and overall poor quality, resulting in texture, density, and color ratings ranging from 4 to 6. Based on the results of physical and chemical soil analyses, and experiment was designed to incorporate site-specific treatments designed to correct mineral deficiencies and bring about improvements in turf texture, density, and color.

MATERIALS AND METHODS

This experiment was conducted during the spring and summer of 2000. Samples were collected from the sixteenth fairway at Fayetteville Country Club. Prior to the establishment of the test plots in June, the entire fairway was plotted with global positioning systems (GPS) equipment and soil test samples were obtained at each coordinate to allow plot mapping. The experimental area was established in a uniform appearing section of the fairway and the individual plots were 1.55 m x 1.55 m with 12 plots (treatments) per block and four blocks (replications) for a total of 48 plots. Soil samples were taken from 60 points within the experimental area. These 60 samples were tested for both chemical and physical properties including texture and bulk density.

The chemical analyses were determined at the University of Arkansas Agricultural Diagnostic Laboratory for macronutrients and micronutrients. Bulk density and particle size analyses were determined in the fairway. Bulk density was determined by taking undisturbed cores of soil and dividing the ovendry mass of soil by the soil volume. Particle size analyses were determined by the methods outlined by Day (1965).

After the bulk density and texture tests were evaluated, bulk density and soil texture was found to be uniform throughout the plot, and thus it was determined that the soil's physical properties were not likely limiting the overall turf quality. All of these measurements were in a uniform and acceptable range. Therefore, using these observations, along with the initial quality ratings, an experiment was designed to evaluate 12 fertility and plant growth regulator combinations to determine if any treatment could improve the color, texture, or density of common bermudagrass.

All treatments were combinations of rates and time of application of commercially available fertilizer products with the exception of Primo™, which is a plant growth regulator that promotes turf density. The experimental design was a randomized complete block with all treatment rates, applications, and descriptions shown in Table 1.

Table 1. Descriptions and rates of fertility and growth regulator treatments.

Treatment	Description and Application
1. Control	No fertilizer, plant growth regulator, or cultural treatments
2. Primo™	Applied monthly (May-September) at 0.05 ai/1000 ft^2
3. Agrilizer™ 100	Slow release N fertilizer (24%N) single application at 2.3 lbs/1000 ft^2
4. Agrilizer™ 200	Slow release N fertilizer (24%N) single application at 4.6 lbs/1000 ft^2
5. Poly O Urea 100	Polyolefin coated slow release urea (40% N) single application at 2.3 lbs/1000 ft^2
6. Poly O Urea 200	Polyolefin coated slow release urea (40% N) single application at 4.6 lbs/1000 ft^2
7. Urea 100 split	Urea (45% N) at 2.3 lbs/1000 ft^2 (split application in June, July, August)
8. Urea 200 split	Urea (45% N) at 4.6 lbs/1000 ft^2 (split application in June, July, August)
9. Nitron™	Natural Organic (9% N) single application at 20 lbs/1000 ft^2
10.Superintendent's choice	Urea in blends (45% N) in split applications at 4.6 lbs/1000 ft^2
11. Urea	(45% N) at 4.6 lbs/1000 ft^2
12. Urea + Mg	(45% N) at 4.6 lbs/1000 ft^2 with MgSO4

*Note: (1) All weed control was standard for all plots. (2) All plots were irrigated at the same time and rate.

All texture, density, and color evaluations were visual and based on the accepted 1 to 9 scale with 9 being most desirable. Texture and density ratings were done in July, August, and September. Color ratings were taken only in September. Analysis of variance was performed on the data to statistically differentiate between treatments and control and Superintendent's choice (JMP4, 2000).

Geostatistics is a statistical tool for determining the distribution of spatial parameters. Geostatistics exploits the spatial relationship of parameters and ultimately enables optimal land management, especially when regarding fertilization. Ca:Mg ratios were computed for the entire sixteenth fairway and analyzed in GS+ a geostatistical software package (Gamma Designs Software, 2000).

No significant differences were observed across the fairway for bulk density and particle size. Soil nutrients were found to be normal with the exception of low soil Mg levels. Evaluation of soil chemical analysis revealed a highly reduced level of soil Mg as expressed by the Ca to Mg ratio (Fig. 1) . The low Ca:Mg values indicated a

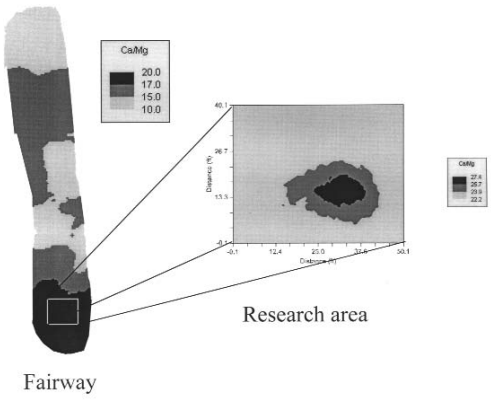


Fig. 1. Kriged map of calcium to magnesium ratio for the fairway and research plot.

Mg deficiency, which could cause poor turf quality and color, since Mg is part of the chlorophyll molecule and is essential for proper nitrogen (N) utilization. If plants do not utilize N properly, poor color and quality often result. Ca to Mg ratios found in our samples ranged from 16:1 to 35:1; much higher than optimal. Optimum Ca:Mg ratio should range from 10:1 to 15:1 (Tisdale, 1993). The location and severity of these deficiencies can be illustrated well in a geostatistical map (Fig. 1).

Analysis of the treated plots showed no visual differences or influence on turf texture. Several of the treatments affected turf color and density. Poly O (100), Poly O (200), Urea 200 and Nitron™ were found to produce higher density ratings in July compared to the control. All remaining treatments showed no influence at all. (Fig. 2). No differences were observed during the second month; however, in the

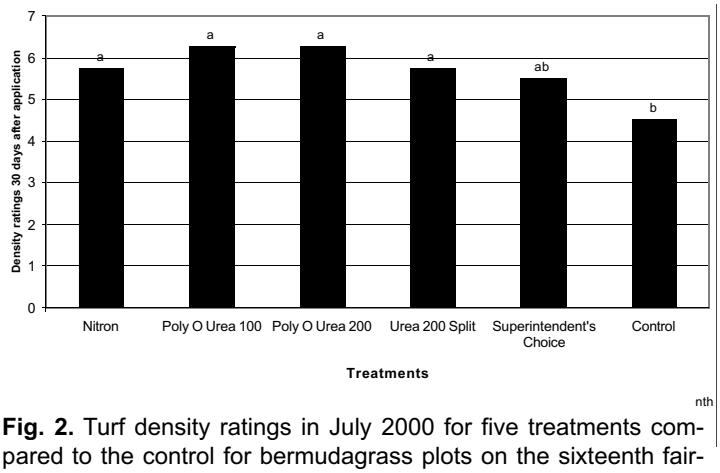


Fig. 2. Turf density ratings in July 2000 for five treatments compared to the control for bermudagrass plots on the sixteenth fairway, Fayetteville Country Club. (P≤0.05)

third month Primo™ and Nitron showed significant differences in turf density when compared to the control (Fig. 3) while other treatments did not. Nitron is a slow release organic source of N, which may explain the delay in having an effect.

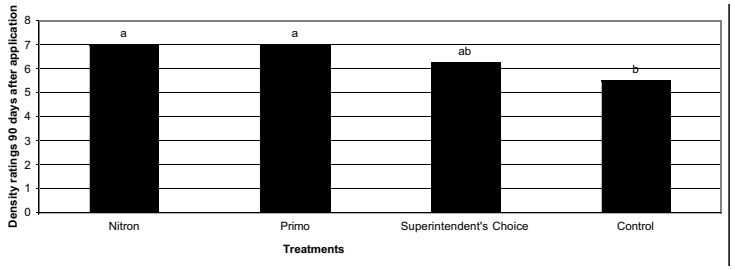


Fig. 3. Turf density ratings in September 2000 for three treatments compared to the control for bermudagrass plots in the sixteenth fairway, Fayetteville Country Club. (P≤0.05)

Plots treated with Primo™ showed significant color change when compared to the Superintendent's choice. Applications of magnesium in the form of $MgSO_4$ were included in an attempt to correct Mg deficiencies found in the soil samples. A significant increase in color ratings resulted from the $MgSO_4$ treatments. In addition

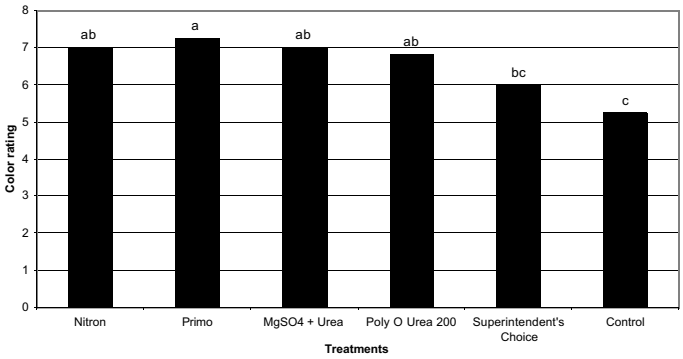


Fig. 4. Final turf color readings in September 2000 for five treatments compared to the control for bermudagrass plots in the sixteenth fairway, Fayetteville Country Club. (P≤0.05)

to $MgSO_4$ treatments, Agrilizer 100, Nitron, and Poly O Urea 200 showed significant increases in color when compared to the control (Fig. 4) while other treatments showed no increase in color.

Kriged maps reveal the spatial distribution of Ca/Mg. Although not used on golf courses at this time, site-specific application of Mg could be used to decrease the Ca/Mg ratio, which would, optimize fertilizer management, and reduce costs, and while improve application efficiency.

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Peripheral blood leukocyte response and macrophage function during *Eimeria adenoeides* infection in turkey poults

Amanda D. Drake,[*] *Sarah E. Heuer,*[*] *Timothy G. Kimball,*[*] *Timothy O. Peters,*[*] *H. David Chapman,*[§] *and Gisela F. Erf*[¶]

ABSTRACT

Intestinal coccidiosis, caused by various species of *Eimeria*, is an economically important disease of chickens and turkeys. The peripheral blood leukocyte response and macrophage functions during a coccidial infection in turkeys have not been defined. To examine these aspects of innate immunity during primary *Eimeria* infection in turkeys, 4-week-old poults were orally inoculated with either 50,000 *E. adenoeides* oocyst (24 infected poults) or water (24 control poults). To monitor the concentrations and proportions of white blood cells (WBC) throughout the course of infection, heparinized blood was collected from 12 infected and 12 control poults prior to inoculation (day 0), and on days 4, 7, and 11 post-inoculation (PI). To study macrophage function, Sephadex-elicited abdominal exudate cells (macrophages) were collected on day 7 PI from 12 infected and 12 control poults. Macrophages were used to study phagocytosis of unopsonized and antibody-opsonized sheep red blood cells (SRBC), production of nitric oxide, and production of cytotoxic factors. *E. adenoeides* infection was associated with alterations in the concentration of WBC, including a decrease in the numbers of circulating lymphocytes on day 4 and a rise in lymphocytes and heterophils on day 11. Although phagocytic activity was not different in macrophages from infected and control poults, macrophages from infected poults exhibited greater cytotoxic activity. Data from these studies strongly suggest that components of innate immunity were recruited and activated during this primary infection of turkey poults with *E. adenoeides*. Further investigations are needed to determine the role of these components in limiting primary infection by *E. adenoeides*.

[*] Freshmen- and sophomore-level students who conducted this interdisciplinary team research project during Spring 2000 as part of the laboratory rotations in the agricultural research course. Amanda Drake is the primary author.

[§] H. David Chapman, teacher and faculty mentor regarding the parasitology aspect of this project, is a professor in the Department of Poultry Science

[¶] Gisela F. Erf, teacher and faculty mentor regarding the immunology aspect of this project, is an associate professor in the Department of Poultry Science.

Meet the Student-Authors

The student authors were first year and sophomore students in the experimental "Laboratory Rotations in Agricultural Research" course funded by a USDA higher education challenge grant. After having learned techniques and approaches used by faculty members David Chapman, Gisela Erf, and Mark Parcells in structured laboratory sessions, this group of students decided to conduct an interdisciplinary team research project combining skills learned in immunology and parasitology under the tutelage of Erf and Chapman. The project culminated in an oral research presentation and a written abstract, describing the objectives, methods, results, and conclusions.

The primary author of this paper is Amanda Drake, who reevaluated the data prior to preparation of the manuscript.

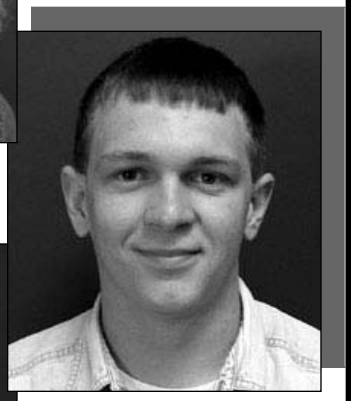
"I thought the class was a really neat experience," Drake said. "When I came to school, I already knew I was interested in doing research, I just didn't know I'd have the opportunity to participate in it so soon." A native of Pine Bluff, and a graduate of Sheridan High School, Drake is now a junior poultry science major.

Amanda D. Drake, Primary Author

Sarah Heuer is a native of Harrison and graduated from Harrison High School. She is now a junior majoring in poultry science.

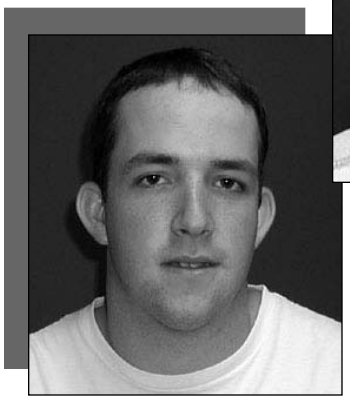
Sarah E. Heuer

Timothy G. Kimball, now a sophomore poultry science major, is from Combs, and graduated from St. Paul High School.



Timothy G. Kimball

Mena native Timothy Peters, a graduate of Mena High School, is a senior poultry science major.



Timothy O. Peters

INTRODUCTION

Coccidiosis is a disease of chickens and turkeys caused by various species of *Eimeria*. The intestinal infection caused by this intracellular protozoan parasite seriously impairs the growth and feed utilization of infected birds, thus coccidiosis has become an economically important disease of poultry throughout the world (McDougald and Reid, 1991). Host immune responses to coccidial infection are complex and not fully understood (Rose, 1996). Although parasite-specific antibodies are produced during the adaptive immune response to *Eimeria*, cell-mediated rather than antibody-mediated responses appear to play an important role in protection against coccidiosis. At this time, direct parasite-specific killing of infected cells by cytotoxic T cells is believed to be a major mechanism of cell-mediated immunity in the elimination of the parasite (Lillehoj and Trout, 1996; McDonald, 1999). During initial exposure to *Eimeria*, components of innate immunity are likely to be important until adaptive immunity has had time to develop. In chickens, immune responses in primary *Eimeria* infections involve changes in white blood cell (WBC) concentrations, production of oxidative radicals including nitric oxide, and production of cytokines such as interleukin-1 and tumor necrosis-like factor (TNLF) (Byrnes et al., 1993; Rose et al., 1979; Zhang et al., 1995). Moreover, macrophages are known to play an important role in reducing oocyst numbers in the feces during a primary infection (Lee and Al-Izzi, 1981).

Eimeria adenoeides develops in the ceca of young turkey poults. Pathological signs of the disease include severe enteritis of the lower small intestine, ceca, and rectum, watery stools (containing mucus or blood), and yellow, cheesy droppings. For *E. adenoeides*, the period from the initial infection to the appearance of oocysts in the feces is between 4 and 6 days (Clarkson, 1958). Depending on the numbers of oocysts ingested (e.g., 100,000 to 200,000), *E. adenoeides* may cause up to 100 % mortality 5 days after infection (Clarkson, 1958). Poults recovering from infection have developed immunity to *E. adenoeides* and the resolution of the infection in the intestine is associated with lymphocyte infiltration into the submucosa and the epithelium of the villi.

The objective of this research project was to examine aspects of innate immunity in response to a primary infection with *E. adenoeides* in 4-week-old turkey poults. Aspects of innate immune activity examined included assessment of WBC concentrations, WBC profiles, and macrophage function over an 11-day period following primary *E. adenoeides* infection.

MATERIALS AND METHODS

Experimental Animals: Forty-eight 4-week-old Nicholas turkey poults were reared at the University of Arkansas Poultry Health Laboratory in a HEPA-filtered environment maintained under biosecurity level 2. At 3 weeks of age, the poults were moved from the Poultry Health Laboratory to a battery cage facility at the University of Arkansas Poultry Veterinary Farm. Two groups of 24 poults were used for these studies; Group 1 was used for a hematology study, and Group 2 was used to study macrophage function. For each group of birds, 12 poults were randomly selected and inoculated orally with 50,000 *E. adenoeides* oocysts (infected birds). The other 12 poults in each group were sham-inoculated with water (control birds). Throughout the duration of the experiments, food and water were available ad libitum and standard rearing, lighting, and temperature protocols were followed. To monitor coccidia infection in Group 1, feces were collected daily from all infected and control poults for determination of the number of oocysts excreted. Similarly, feces from Group 2 were collected and oocyst counts conducted on a daily basis until birds were euthanized on day 7 post-inoculation (PI). Additionally, poults in Group 2 were subjected to post-mortem examination to determine the severity of coccidia infection based on a lesion score that ranged from 0 (no lesion) to 5 (maximal lesion).

Hematology Study: Prior to and 4, 7 and 11 days PI, all poults in Group 1 were weighed to the nearest gram, and a 1.5 mL blood sample was taken from a wing vein using a heparinized syringe. Total WBC concentration was determined using an automated hematology analyzer (CELL-DYN). Blood smears were also prepared and stained with Wright stain to determine the proportions of the various WBC populations (lymphocytes, heterophils, monocytes, eosinophils, and basophils). For each poult, at least 300 WBC were examined using a bright field microscope and 1000x magnification (Lucas and Jamroz, 1961). The concentration of each type of WBC was then calculated based on the total WBC concentration and on the proportion of a type of WBC within the total WBC population. Body-weight

measurements were used to calculate total body-weight gain over the 11-day experimental period (day 0-11), body-weight gain between day 4 and day 11 (day 4-11), and body-weight gain between day 7 and day 11 (day 7-11).

Abdominal-Exudate Cell Elicitation and Preparation of Macrophage Cell Suspensions: Five days PI, birds in Group 2 were weighed and injected intra-abdominally with a 3% solution of Sephadex (G-50) beads (1 mL/100 g body-weight). Forty-two to 44 hours post-Sephadex injection, the birds were euthanized with pentobarbital and the abdominal exudate cells were harvested. Abdominal exudate cells (macrophages) were then washed with Dulbecco's phosphate-buffered saline (PBS) and the cell concentrations were adjusted to 4 x 106 cells/mL with LM Hahn medium. Macrophages were used to determine phagocytosis as well as production of soluble factors such as nitric oxide (nitrite assay) and TNLF (cytotoxicity assay).

Phagocytosis: Macrophages from each poult were allowed to adhere to glass coverslips during a 45-min incubation at 41ºC. Glass coverslips with adherent macrophages were then incubated with sheep red blood cells (SRBC) or with antibody-opsonized SRBC (Ab-SRBC) for 45 minutes at 41ºC. After incubation, the coverslips were washed with PBS, stained with Wright stain, and placed on microscope slides (one for SRBC and one for Ab-SRBC per poult). For each slide, 900 macrophages were examined using a microscope. The numbers of macrophages with and without internalized SRBC as well as the number of SRBC within a phagocytically active macrophage were recorded.

Nitrite Assay and Cytotoxicity Assay: To assess the release of soluble factors by macrophages, macrophages were plated in 24 well culture plates (2 x 106 cells/well) and incubated with and without E. coli LPS (10 mg/culture) for 24 hours at 37ºC with 5% CO_2. Following incubation, the supernatant fluid was collected. For the nitrite assay, 100 mL of each supernate were plated in duplicate in 96-well plates. Greiss reagent was added to each of the wells and the plates were read at 540 nm with an automated microplate reader. Standard concentrations of nitrite ranging from 1.25 mM to 90 mM were included in each plate to establish the relationship between nitrite concentration and absorbance units (a.u.). The equation describing the linear relationship between a.u. and nitrite concentration was then used to determine the concentration of nitrite produced by unstimulated and LPS-stimulated macrophages from infected and control poults.

For the cytotoxicity assay, 50 mL of each macrophage supernate were added to 2 x 106 RP-9 cells/well (tumor cell line) in 96-well culture plates. The cultures were then incubated for 18 hours at 37ºC with 5% CO_2 . Following incubation, MTT colorimetric assay was used to detect surviving RP9 cells (Mosmann, 1983). The plates were read at 540 nm with an automated microplate reader. RP9 cells incubated with medium alone or with a solution of 0.02% Triton-X (detergent) were used as the negative (no cytotoxicity) and positive (complete cytotoxicity) controls, respectively.

Statistical Analyses: For each aspect examined in this study, data were analyzed for the effect of treatment by one-way ANOVA using the Systat Statistical Analysis software (SPSS Inc., Chicago, Ill.). Data were presented as means ± SEM. Differences between means with a P-value of ≤0.05 were considered significant.

RESULTS AND DISCUSSION

Pathology of Infection: In *E. adenoeides*-infected poults from Group 1, oocysts were first recovered in the feces on day 5 PI (4 x 106/bird). The number of oocysts recovered in the feces of Group 1 poults was highest on day 6 PI (20.2 x 106/bird), decreased to 14.7 x 106/bird by day 9 PI, and then dropped drastically to 1.4 x 106/bird by day 11 PI. No oocysts were recovered from the feces of controls throughout the 11-day study. Total weight gained by infected poults between day 0 and day 11 PI tended to be less than that of controls (Table 1). However, day 4-11 and day 7-11 body-weight gain of infected poults was significantly (P

Table 1. Body weight gain (g) in turkey poults inoculated with *Eimeria adenoeides* or water.

Growth period	Body-weight gain (mean ± SEM) Infected	Control
Day 0 - 11 post-infection[z]	745.7 ± 59.1	847.5 ± 38.8
Day 4 - 11 post-infection	414.3 ± 30.4 **b**[,y]	542.8 ± 26.2 **a**
Day 7 - 11 post-infection	231.7 ± 20.3 **b**	354.8 ± 17.1 **a**

[z] When the poults were 4 weeks old, 12 poults were infected with 50,000 *Eimeria adenoeides* oocysts/poult administered orally; 12 poults were uninfected (controls).

[y] Different letters within a row indicate significant (P < 0.05) differences between infected and control poults.

< 0.05) lower than that of controls (Table 1). The reduction in body-weight gain of infected poults may be explained by the pathogenic nature of the *Eimeria* infection, which resulted in lesions and associated enteritis of the lower small intestine, ceca, and rectum, and impaired digestion and absorption of food. The presence of lesions in infected poults was confirmed when poults from Group 2 where euthanized on day 7 PI and lesion scores were determined. In this group of birds, as in Group 1, oocysts were first recovered from the feces on day 5 PI (9.1 x 106/bird), with higher numbers of oocysts recovered on day 6 and 7 PI (27.6 and 25.0 x 106/bird, respectively). At termination of the macrophage function study (day 7 PI, Group 2), all infected poults had developed cecal lesions with lesion scores ranging between 1 and 4 on a 0 to 5 point scale. Lesion scores in poults from the control group were zero.

In summary, the large number of oocysts isolated from the feces, the reduction in body-weight gain, and the lesions observed in infected poults attest to the success of the induction of coccidiosis in all poults that had been infected orally with *E. adenoeides* oocysts.

Effect of *E. adenoeides* Infection on the Concentrations and Proportions among White Blood Cells: The *Eimeria* infection in the gastrointestinal tract resulted in altered concentrations of WBC (Table 2). Compared to controls, infected poults had reduced (P = 0.09) concentrations of WBC on day 4 PI, and

Table 2. White blood cell (WBC) concentrations (x103/mL) in blood from turkey poults inoculated with *Eimeria adenoeides* or water.

	WBC concentration (mean ± SEM)		
Time[z]	Infected	Control	P-value
Day 0	42.31 ± 3.26	40.05 ± 2.97	0.631
Day 4	35.11 ± 1.39	42.38 ± 3.86	0.090
Day 7	40.28 ± 1.93	45.92 ± 3.93	0.212
Day 11	46.39 ± 3.02 [a, y]	37.14 ± 1.86 [b]	0.015

[z] When the poults were 4 weeks old, 12 poults were infected with 50,000 *Eimeria adenoeides* oocysts/poult administered orally; 12 poults were uninfected (controls).

[y] Different letters within a row indicate significant (P < 0.05) differences between infected and control poults.

increased (P < 0.05) concentrations of WBC on day 11 PI (Table 2). The reduction in the concentration of WBC detected on day 4 PI was primarily due to lower levels (P < 0.05) of lymphocytes in infected poults compared to levels in poults from the control group (Table 3). The increase in WBC concentrations in infected poults compared to poults from the control group was due to an increase (P < 0.05) in both the number of lymphocytes and the number of heterophils (Table 3). Similar observations have been made in broiler chickens where a drop in the concentration of blood lymphocytes was associated with maximal output of oocysts in the feces (Rose et al., 1979). The drop in blood lymphocyte concentrations observed here also coincided with maximal excretion of oocysts in the feces. Based on histological examination of intestinal tracts from chickens and turkeys (Clarkson, 1958; Rose et al., 1979, 1984) infected with *Eimeria*, large infiltrations of lymphocytes into the intestinal submucosa and epithelial tissues occurred during the same period of time following primary infection. Hence, the drop in the amount of WBC can be explained by the migration of lymphocytes from the blood to infected tissues. The elevated concentrations of blood lymphocytes and heterophils on day 11 PI are in accordance with a similar rise observed in *Eimeria* infected rats and chickens (Rose et al., 1979) which has been attributed to the establishment of a protective response and the resolution of infection. The elevated eosinophil concentrations on day 4 PI and reduced monocyte concentrations on day 11 PI observed in the blood of infected poults can also be explained by recruitment of these cell types to the site of infection. Eosinophils constitute a first line of defense against large parasites. Similarly, monocytes, called macrophages after they leave the blood to enter other tissues, are cells that are specialized in killing intracellular parasites. As reported by Clarkson (1958), eosinophils were present in large numbers in the cecal submucosa of turkeys with *E. adenoeides* infection on day 1-6 PI, whereas, macrophages were most abundant in infected tissues during the resolution of the *Eimeria* infection (Rose et al., 1979). Overall, the alterations in the concentrations of WBC in *E. adenoeides* infection in turkeys are similar to those reported for *Eimeria* infections in other species and can be explained by altered production and recruitment of cells required to resolve the infection and develop protective immunity to the infective agent.

Effect of *E. adenoeides* Infection on Macrophage Function: An important role of macrophages in primary infection with *Eimeria* became apparent when the number of oocysts excreted in the feces of infected chickens was four times higher when their macrophages had been selectively killed in vivo (Lee

Table 3. Concentrations and proportions among white blood cells in turkey poults inoculated with *Eimeria adenoeides* or water.

Day[z]	Treatment	Lymphocytes	Heterophils	Monocytes	Eosinophils	Basophils
Concentration (# of cells/mL of blood) [y]						
0	infected	23.47 ± 2.09	14.43 ± 2.04	1.92 ± 0.43	0.56 ± 0.15	2.14 ± 0.49
0	control	21.61 ± 1.66	13.79 ± 1.50	1.98 ± 0.24	0.54 ± 0.16	2.18 ± 0.49
4	infected	19.64 ± 0.57 **b**,[x]	11.59 ± 1.29	1.79 ± 0.32	0.46 ± 0.12 **a**	1.73 ± 0.30
4	control	22.13 ± 1.31 **a**	15.18 ± 2.24	2.51 ± 0.38	0.17 ± 0.04 **b**	1.93 ± 0.33
7	infected	22.64 ± 0.19	11.53 ± 1.60	3.20 ± 0.48	0.67 ± 0.15	2.29 ± 0.31
7	control	21.90 ± 1.96	13.39 ± 1.00	4.03 ± 0.53	0.46 ± 0.13	2.44 ± 0.40
11	infected	22.56 ± 1.39 **a**	18.99 ± 2.88 **a**	2.32 ± 0.22 **b**	0.65 ± 0.16	1.61 ± 0.20
11	control	18.76 ± 0.98 **b**	12.07 ± 1.10 **b**	3.56 ± 0.33 **a**	0.33 ± 0.07	1.37 ± 0.20
Proportions (% of total leukocytes) [w]						
0	infected	52.82 ± 1.83	33.78 ± 3.53	4.41 ± 0.76	1.35 ± 0.37	5.43 ± 1.56
0	control	52.45 ± 2.51	34.08 ± 2.85	5.02 ± 0.58	1.36 ± 0.46	4.93 ± 0.87
4	infected	56.76 ± 2.36	32.30 ± 2.70	4.96 ± 0.82	1.29 ± 0.31a	4.95 ± 0.83
4	control	52.49 ± 2.47	34.54 ± 2.94	5.79 ± 0.64	0.48 ± 0.15b	4.52 ± 0.63
7	infected	56.24 ± 4.01	28.50 ± 3.67	7.84 ± 1.04	1.63 ± 0.36	5.28 ± 0.74
7	control	52.24 ± 3.43	34.02 ± 3.32	8.71 ± 0.86	0.99 ± 0.26	5.07 ± 0.63
11	infected	51.09 ± 3.89	38.05 ± 4.06	5.63 ± 0.55 **b**	1.32 ± 0.26	4.10 ± 0.63
11	control	53.35 ± 2.32	32.35 ± 2.42	9.62 ± 0.81 **a**	0.86 ± 0.17	3.77 ± 0.59

[z] When the poults were 4 weeks old, 12 poults were infected with 50,000 *Eimeria adenoeides* oocysts/poult administered orally; 12 poults were uninfected (controls).

[y] For each blood sample, total leukocyte concentration was determined using an automated hematology analyzer (CELL-DYN). The concentration of various leukocyte populations was then calculated using total leukocyte concentration and the manual estimate of the proportion of each cell type (see footnote 3).

[x] Different letters within a day and cell type indicate significant ($P < 0.05$) differences between infected and control poults.

[w] For each blood sample, the proportion among leukocyte populations was estimated by identifying 300 leukocytes within a Wright-stained monolayer of blood cells using a bright-field microscope (1000 x magnification).

Table 4. Macrophage phagocytic activity in turkey poults inoculated with *Eimeria adenoeides* or water[z].

Activity[y]	Infected	Control
Percentage of macrophages phagocytozing unopsonized SRBC	9.53 ± 0.99	8.81 ± 1.27
Percentage of macrophages phagocytozing Ab-opsonized SRBC	82.09 ± 3.62	83.46 ± 4.02
Number of unopsonized SRBC phagocytozed per phagocytic macrophage	1.49 ± 0.18	1.63 ± 0.22
Percentage of Ab-opsonized SRBC phagocytozed per phagocytic macrophage	3.16 ± 0.16	3.09 ± 0.14

[z] When the poults were 4 weeks old, 12 poults were infected with 50,000 *Eimeria adenoeides* oocysts/poult administered orally; 12 poults were not infected (controls).

[y] Five days post-infection, Sephadex beads were injected into the abdominal cavity of 12 infected and 12 control poults. Sephadex-elicited abdominal macrophages were harvested 42 hours later and incubated with SRBC or antibody-opsonized SRBC. For each activity, 900 macrophages per bird were examined using a microscope.

and Al-Izzi, 1981). Additionally, factors such as nitric oxide and TNLF produced by macrophages, have been shown to be important in the reduction of oocyst excretion during primary *Eimeria* infection, further supporting an important role of macrophages in coccidiosis. On day 7 PI, macrophages obtained from infected and control poults had similar abilities to carry out lower-order functions, including phagocytosis of unopsonized SRBC and Fc-receptor-mediated phagocytosis of antibody-opsonized SRBC (Table 4). Although macrophage production of nitric oxide was not affected by *E. adenoeides* infection (Fig. 1), nitric oxide production by LPS-activated macrophages from infected poults tended to be higher (P = 0.121) than

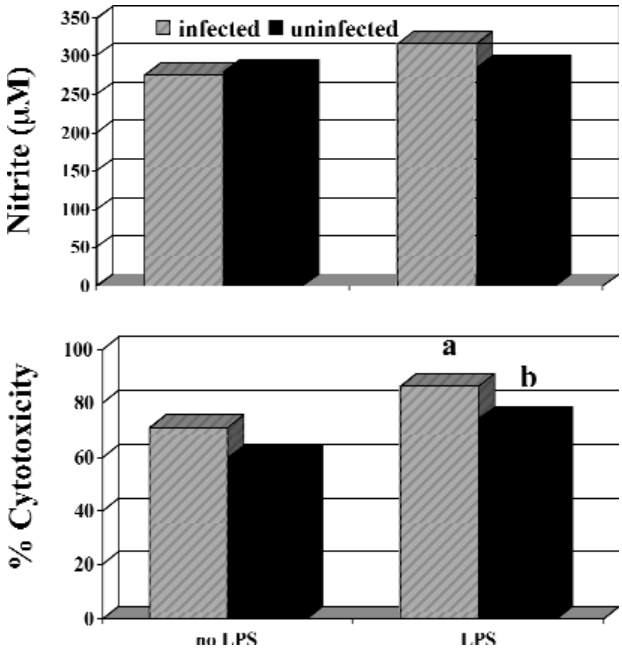


Fig. 1. Nitric oxide production (mM) and cytotoxicity (%) by macrophages from turkey poults inoculated with *Eimeria adenoeides* or water at 4 weeks of age. Abdominal exudate cells (macrophages) were elicited from 12 infected and 12 uninfected poults on Day 5 post-infection by injection of Sephadex beads into the abdominal cavity. Forty-two hours later, macrophages were harvested and cultured with or without lipopolysaccharide (LPS). Culture supernatant fluid from LPS-activated and unactivated macrophage cultures was collected 24 hours later. Culture supernatants were assayed for nitric oxide production and cytotoxicity by nitrite assay and killing of RP9 tumor cells, respectively.

that by LPS-activated macrophages from controls. Similarly, the production of cytotoxic factors (i.e., TNLF) by macrophages from infected poults tended to be higher than that from controls, although this trend was only significant ($P < 0.05$) when macrophages were further activated in vitro with LPS (Fig. 1). These differences in higher-order functions of macrophages, especially in the production of cytotoxic factors, suggest that the internal environment of infected birds already provides signals for macrophages to become more responsive to stimuli (e.g., LPS, *Eimeria*). Nitric oxide and cytotoxic factors like TNLF have been shown to be beneficial during primary *Eimeria* infection in chickens, although TNLF tended to exhibit both protective and pathological effects (Allen and Lillehoj, 1998; Byrnes et al., 1993; Zhang et al., 1995). Pathological effects of TNLF included primarily metabolic effects such as body-weight reduction, whereas, TNLF did not appear to contribute to the intestinal lesions observed during *Eimeria* infections (Zhang et al., 1995). Overall, macrophages obtained from poults with primary *E. adenoeides* infection exhibited a higher level of responsiveness to LPS stimulation than macrophages from controls, suggesting a priming effect of the internal environment in infected birds. This heightened responsiveness to environmental stimuli (e.g., signals from components of adaptive immunity) and the resulting increase in macrophage activity are likely to be important in the resolution of *E. adenoeides* infection.

In summary, data from these studies strongly suggest a response to an initial *E. adenoeides* infection by components of innate immunity in infected turkey poults.

ACKNOWLEDGMENTS

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Prediction of rice texture from starch profiles measured using high-performance liquid chromatography

Hazel Fromm[*] *and J.-F. Meullenet*[§]

ABSTRACT

Starch determines a large proportion of the textural properties of cooked rice. The amylose: amylopectin ratio plays a significant role in the functionality of native starch. In this study a medium-grain rice cultivar, 'Bengal', was used for starch structure characterization using high performance size-exclusion chromatography (HPSEC). This cultivar is characterized by having lower amylose content (15% to 20%) than long grain cultivars and being sticky when cooked, similar to short-grain cultivars. Rice samples were harvested in 1999 from five locations around Arkansas at state verification trials where cultural practices are closely monitored. Samples of this cultivar stored at a specified moisture level for a pre-determined period of time were also subjected to texture analysis by means of a Texture Analyzer. The data measured with the HPSEC was related to instrumental texture attributes. Chemical characterization data (carbohydrate profiles) of rice samples were used to predict texture attributes of cooked rice such as hardness and stickiness. Instrumental texture attributes of hardness and stickiness were successfully predicted for Bengal rice from starch-profile data obtained though HPSEC analyses. Both attributes proved to be well predicted, based on their high coefficients of determination of 0.97 and 0.85, respectively. The statistical analysis indicates that starch structure characterization using HPSEC may be related to instrumental measurements of texture attributes.

[*] Hazel Fromm graduated in May 2001 with a degree in food science.

[§] J.-F. Meullenet, faculty sponsor, is an assistant professor in the Department of Food Science.

Meet the Student-Author

Hazel Fromm

I graduated in May 2001 as a Senior Scholar with a major in food science and a minor in agricultural business. I transferred to the U of A in 1999 after earning a degree as an agronomist from the Zamorano Pan American School of Agriculture in Honduras. I was awarded the Foundation for the International Exchange of Students (FIES) scholarship to attend the University of Arkansas. My two years at the U of A have been an exciting learning experience, both inside and outside the classroom.

Since my arrival at the U of A, I have been able to gain practical experience in my field of study outside the classroom by working in different laboratories at the Department of Food Science. I have worked in the Sensory Laboratory, the Rheology Lab, and the Pickle Lab. Through this exposure and hands-on work, I have been able to determine which area I would like to specialize in as I advance into graduate studies. I am particularly interested in sensory science. By means of my undergraduate research I was able to acquire laboratory and research skills that will definitely prove to be useful throughout my graduate education.

My co-curricular activities include the Food Science Club, of which I served as president, member of the Institute of Food Technologists Student Association, Gamma Sigma Delta and Golden Key Honor Societies, and Bumpers College Ambassador. I had the opportunity do a summer internship abroad at the Scottish Agricultural College. I was able to carry out research related to my field of study, experienced from an international perspective.

I was an intern at Nestlé in York, England, in summer of 2001—working on textural analysis of low-calorie chocolate. Beginning in fall 2001, I will be attending graduate school at Cornell University, where I plan to specialize in dairy and sensory science.

INTRODUCTION

Starch is the principal component of rice and is made up mainly of a mixture of the polysaccharides amylose and amylopectin. Typically starches contain 20-30% amylose and 70-80% amylopectin. Starch is a polymer of glucose and an alpha-glucan containing mainly alpha 1,4-glucosidic linkages with smaller alpha 1,6-glucosidic linkages forming branch points (Pomerzanz, 1971). Starch dictates a large proportion of textural properties of cooked rice (Hamaker, 1999). The amylose: amylopectin ratio plays an important role in the functionality of native starch. Starch characteristics such as viscosity, gelatinization, and texture are functions of the amylose:amylopectin ratio (Satin, 2000). Structure of the amylose and amylopectin molecules, or their degree of polymerization, will also affect starch structure and function (Hegenbart, 1996).

Several physical characteristics of starch granules have an impact on its functionality. These characteristics include size, shape, surface, and distribution of starch granules (Satin, 2000). The organization of starch granules can be greatly influenced by genotype and environmental conditions (Smith et. al., 1997). This result can be attributed to the various isoforms of starch synthase, an enzyme responsible for the forma-

tion of amylopectin (Priess and Sivak, 1996). Starches may be altered physically, chemically, or enzymatically in order to improve functional properties (Lineback, 2000).

'Bengal'- a medium-grain rice cultivar, has lower amylose content and is sticky when cooked, similar to short-grain cultivars (Uebersaz, 2000). The amylose content of medium grain rice ranges from 15% to 20% (Webb et. al., 1979). According to Juliano et. al. (1981), variation in amylose content of milled rice is a factor that considerably affects the texture and thus the cooking and eating qualities of rice.

Investigators from the University of Arkansas Rice Processing Program have observed that the existing medium-grain cultivar, Bengal, seems to be more susceptible to variability in functionality than long-grain cultivars. For this reason, they hypothesized that not all cultivars present the same degree of susceptibility to quality inconsistency. If specific physical and chemical characteristics that are linked to high functional variability can be identified, this information could be useful to rice breeders in producing rice that is not as susceptible to functional variability as existing cultivars are.

The objectives of the study were to (1) characterize starch structure using High Performance Size-Exclusion Chromatography (HPSEC) and relate the data obtained to instrumental texture attributes, and (2) use chemical characterization data (carbohydrate profiles) of rice samples to predict texture attributes of cooked rice such as hardness and stickiness.

MATERIALS AND METHODS

Rice Samples. Bengal rice was used for this study. Rice samples were harvested in 1999 from five locations around Arkansas at state verification trials where cultural practices are closely monitored. The locations were the following: (Bengal A) Mississippi County; (Bengal B) Cross County; (Bengal C) Greene County; (Bengal D) Prairie County; and (Bengal E) Mississippi County, in the same field as BA but after a heavy rain prior to harvest (5.44 cm on 29 Sept. 1999). All locations are privately owned, with the exception of Mississippi County location, which is part of the North East Research and Extension Center (NEREC). Harvested rice was transported to the Rice Processing Laboratories at the Food Science Department, University of Arkansas, Fayetteville, where it was cleansed and dried in a laboratory drier at 33ºC and 67.8% relative humidity (low temperature drying).

The samples were then subjected to several post-harvest treatments, including assessment of equilibrium moisture content and storage duration. The rough-storage moisture contents for all 15 samples were equilibrated to 12% in an equilibrium chamber at 21ºC. Finally the samples were stored at 21ºC and sampled at 0, 12, and 24 weeks. The samples from each location were then stored in a freezer until further chemical and textural analyses were conducted. The moisture content of the samples after extended storage (approx. 16 months) ranged from 8% to 16%, depending on location and storage time. Bengal rice samples at 0 weeks had the lowest moisture content, averaging 9.5% across the five sampled locations.

Starch Isolation. Rice starch was isolated using a modified alkaline method (Hoover and Sosulski, 1985). A 0.2% NaOH solution was used to extract the starch. The dried starch cake that resulted was ground and stored at -20ºC. The isolated rice starch obtained was then used to prepare the defatted starch samples for high-performance liquid chromatograph (HPLC) injection. Defatted starch was obtained by pipetting 10 mL of butanol for every gram of rice starch. Samples were left shaking (LabQuake, Barnstead/Themolyne, Dubuque, Iowa) overnight. Samples were collected into aluminum pans and left to dry.

Starch samples for HPLC injection were prepared by adding 5 mL of 90% methylsulfoxide to 20 mg of defatted rice starch. Samples were placed in a water bath for 1 hour and stirred overnight. Finally, the samples were filtered through a 5-µm Waters brand membrane.

Instrumental Texture Analysis. Instrumental texture analysis of the samples was carried out using a Texture Analyzer (Model TAXT2i, Texture Technologies Corp., Scarsdale, N.Y.) equipped with Texture Expert data acquisition software (Version 1.22, Stable Microsystems, Surrey, England). A compression fixture (100-mm diameter compression plate) and base plate were required for the double compression test. A 50-kg load cell was used with the instrument, allowing a return distance of 30 mm.

Sample preparation for instrumental texture analysis required samples to be cooked for 30 minutes in a rice cooker under steam conditions (Aroma Rice Cooker/Steamer, Model ARC-707, San Diego, California). Ten grams of milled rice were placed in a 100 ml beaker and combined with 17 g of water. Once water in the rice cooker (350 ml) was boiling, the sam-

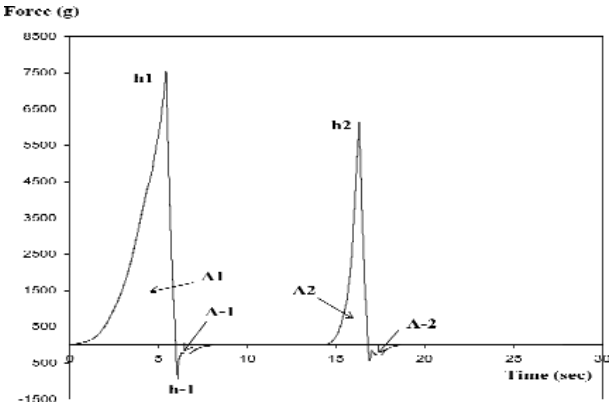
ple was placed on a rack in the center of the cooker to prevent direct contact with the heated surface. After 30 minutes, the rice cooker was turned off and the rice remained in it for 5 minutes. Ten rice kernels were then placed on the surface of a clean aluminum base plate and analyzed. Two cooking replications were performed for each sample and six measurements were made for each cooking replication. Sample temperature was monitored closely as lower temperature instantly affects texture and would therefore produce inaccurate, unreliable measurements (Meullenet et. al., 1998, 1999).

The crosshead speed of the texture analyzer was set at 5 mm/second and the deformation to 90% of each sample's original height. A complete texture profile analysis was then obtained from the Texture Expert software. The software recorded force-distance curves for the double compression. The software was used to write and run a macro for each sample, with the purpose of calculating values for instrumental texture attributes. Calculated parameters included texture attributes such as hardness and stickiness (Fig. 1).

Starch Structure Characterization. The samples were analyzed for carbohydrate profiles by HPSEC according to methods developed by Wang and Wang (2000). Native starch was separated using a series of Shodex OHpak columns maintained at 55ºC with a column heater. Components of the HPSEC used to determine the amylose: amylopectin ratio included a 515 HPLC pump and an injector with a 100 µl sample loop, an inline degasser, and a refractive index detector. The previously mentioned equipment was used along with a high performance anion-exchange chromatograph with pulsed amperometric detection (Wang and Wang, 2000). An aqueous solution of $NaNO_3$ and NaN_3 was used as the mobile phase. Dextran polymers of various molecular weights were used as standards. The data were obtained from one replication of each rice sample.

Statistical Analyses. Chemical characterization data obtained from the HPLC analyses were used for prediction of corresponding functional attributes such as sensory texture and cooking properties. Mathematical models using multivariate analysis techniques were used to develop a predictive model (Meullenet, et al., 1999).

The statistical methods used were partial least squares regression and principal component analysis using the Partial Least Squares (PLSI) and Principal Component Analysis (PCA) options in The Unscrambler software (version 7.5, CAMO ASA, Throndheim, Norway, 1996).



Variable description	*Variable name*
Sample height at the beginning of the test	Height2
Maximum force for the first positive peak (first compression cycle)	h1
Minimum force for the first negative peak	h1
Maximum force for the second positive peak (second compression cycle)	h2
Area under the first positive peak curve	A-1
Area under the first negative peak curve	A-1
Area under the second positive peak curve	A-2
Area under the second negative peak curve	A-2
Ratio A2/A1	Cohesiveness

Fig. 1. Typical TPA test curve and instrumental parameters extracted from the force/deformation curves for TPA test.

RESULTS AND DISCUSSION

Instrumental texture attributes of hardness (h1) and stickiness (A-1) were successfully predicted for Bengal rice from starch-profile data obtained though HPSEC analyses. Both attributes proved to be well predicted as can be observed from their high coefficients of determination of 0.97 (Fig. 2) and 0.85 (Fig. 4), respectively.

The force versus time curve presented in Figure 1 was used to determine the value of the two instrumental texture parameters analyzed. Hardness can be defined as the maximum force during the first compression (H), while stickiness is the area under the first negative-peak curve (A-1).

The starch profile data were superimposed to the weighted regression coefficients for

instrumental hardness (Fig. 3). As the graph shows, the first peak indicated amylopectin, which is of higher molecular weight than amylose. Since the process was one of size exclusion, this peak appeared before the amylose peak, which is a lower molecular- weight structure. The textural data indicate that higher relative concentrations of amylopectin were responsible for a lower hardness value. It was also found that an increase in low molecular-weight amylose resulted in an increase of Bengal rice hardness. Juliano et. al. (1981) found amylose content to be positively correlated with the hardness value and negatively correlated with stickiness.

Starch profile data were also superimposed on the weighted regression coefficients for instrumental stickiness (Fig. 5). In this graph, the relation between high levels of amylopectin and stickiness is positive. The peak indicating presence of the higher molecular-weight amylopectin obtained from HPSEC analysis coincides with an increase in adhesiveness. The superimposed curve of regression coefficients for stickiness begins to decline sharply at the same point at which the amylose peak begins to rise. The relationship between amylose presence and the stickiness attribute is therefore inverse.

It is relevant to point out that the HPSEC equipment used for sample analysis was not functioning at optimal conditions. This was due to deterioration of the column, and which may have caused an irregular flow of the liquid that continuously carries the sample from the top to the bottom of the column.

The statistical analysis presented indicates that starch structure characterization using HPSEC may be related to instrumental measurements of texture attrib-

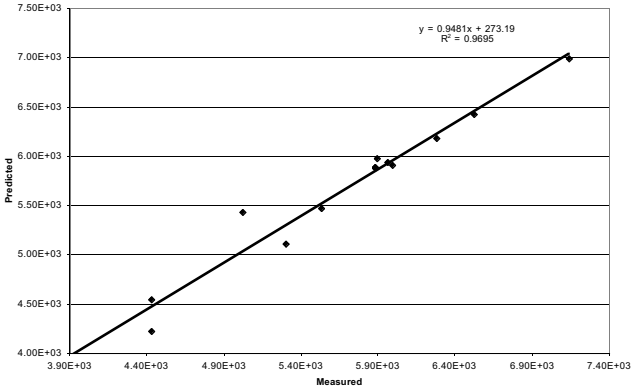


Fig. 2. Measured versus predicted hardness from HPLC data.

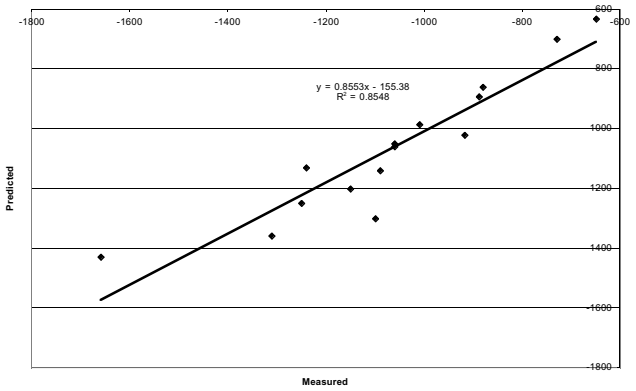


Fig. 4. Measured versus predicted stickiness from HPLC data.

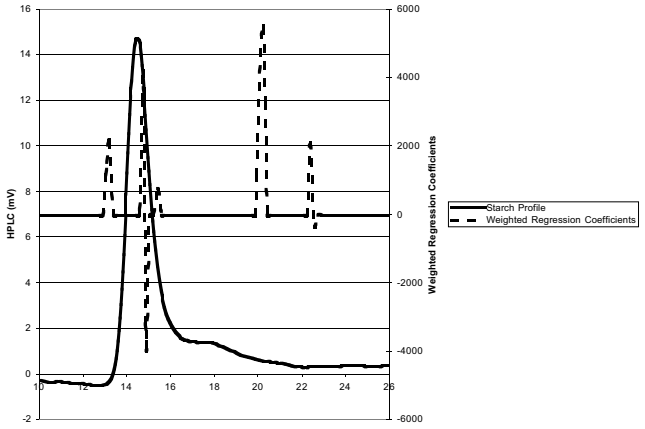


Fig. 3. Prediction of instrumental hardness (h1) from starch profile data.

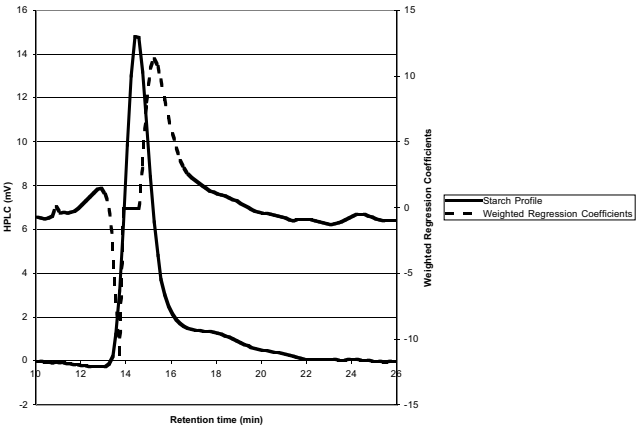


Fig. 5. Prediction of instrumental stickiness (A-1) from starch profile data.

utes. Instrumental hardness and stickiness were successfully predicted for Bengal rice from starch-profile data. Both attributes proved to be well predicted as demonstrated by their high coefficients of determination of 0.97 and 0.85, respectively. Further research is necessary to determine if these attributes can be accurately predicted for different rice cultivars based on their individual carbohydrate profile.

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Correlating fissure occurrence to rice quality for various drying and tempering treatments

M.J. Jiménez,[*] *T.J. Siebenmorgen,*[§] *and A.G. Cnossen*[¶]

ABSTRACT

When a rice kernel fissures, it can break in subsequent food processing operations and lose its commercial value. Head rice yield (HRY) is a measure of the percent of kernels that remain whole (at least three-fourths of original length) after rice has been milled. Our experiment was designed to test the effect of a rapid state transition during drying and tempering processes using cultivars Bengal and Cypress. 'Bengal' is a medium-size kernel and 'Cypress' is a long-size, thinner grained cultivar. Immediately after drying, the rice samples were separated into four sub-samples and tempered for 0, 80, 160, or 240 minutes at the temperature of the drying air. Tempering is a process to allow kernel moisture content gradients to decrease, thereby reducing the stress within the kernel. From each sample, 400 kernels were randomly selected, visually observed, and the percentage of fissured kernels determined. Results showed that the percentage of fissured kernels generally decreased with tempering. However, some samples still showed many fissures even after extended tempering, yet had a high HRY. While HRY is currently the primary index of rice quality, it is known that fissured kernels can severely and detrimentally affect end-use processing operations such as cooking or puffing. Thus, the tempering duration required for preventing kernel fissuring might be longer than the tempering duration required for maintaining a high HRY.

[*] Mónica J. Jiménez is studying chemical engineering in the Department of Food Science.

[§] Terry J. Siebenmorgen, faculty sponsor, is a professor in the Department of Food Science.

[¶] A. G. Cnossen is a research specialist in the Department of Food Science.

Meet the Student-Author

I am a senior in chemical engineering at the University of Arkansas. I am originally from Mexico City but went as an exchange student to Canada for high school and then started my undergraduate work here. I have received several awards and scholarships and I am member of national societies such as Golden Key.

In the Food Science Department I have been given the opportunity to work in research of rice. I have found this experience enormously beneficial. It has given me skills in scientific procedures and broadened my trains of thought. I have also become more aware of the importance of this discipline in which my major knowledge can be applied.

I plan to join the workforce after my graduation in December 2001 so I can gain enough experience to decide which career I want to pursue and then maybe obtain a master's degree.

Monica Jimenez

INTRODUCTION

Rice kernel fissuring and breakage is a major problem in the rice industry. Fissured kernels will cause HRY reduction and thus decrease the value of the rice crop. Broken kernels are typically worth approximately half the value of whole kernels. Kunze and Hall (1965) stated that a rice kernel with two or three cross-sectional fissures has lost its commercial value.

The main component of rice is starch, which has, like other polymers, a glass transition temperature (Tg). According to the glass transition hypothesis, when heated above Tg, the kernel changes from a "glassy" to a "rubbery" state. During this transition, the material properties change dramatically which can result in fissuring.

Several researchers (Kunze, 1979; Nguyen and Kunze, 1984) have found that kernels do not fissure until after the drying process has ceased. Previous research (Cnossen et al., 1999) on drying and tempering of rice, conducted by the University of Arkansas Rice Processing Program, concluded that high drying air temperatures can be used without incurring HRY reduction as long as proper tempering techniques are used. High tempering temperatures were shown to be very effective in maintaining high HRY. However, Siebenmorgen et al. (1998) and Matthews et al. (1970) concluded that some fissured kernels would not break during the milling process and remain as head rice. During further processing (cooking, puffing, etc.) these kernels may break and reduce the quality of the final product.

An understanding of the effect of various drying and tempering treatments on fissure occurrence and the relation between fissures and breakage will provide end-users, such as cereal and cooked-rice product manufacturers, with information to optimize their processing operations. Because of the paramount importance of milling quality and kernel physical quality, understanding this relationship would greatly improve the value of rice, and thus the sustained profitability of rice production.

The objectives of this study were to: 1) determine the effect of various drying and tempering treatments on fissure occurrence based on the glass transition hypothesis and 2) correlate fissure occurrence data and HRY data to determine optimum drying conditions and minimum tempering durations to maximize both HRY and kernel physical integrity.

MATERIALS AND METHODS

The cultivars Bengal (medium-grain) and Cypress (long-grain) at two harvest moisture contents (HMC), high (19% to 20%) and low (17% to 18%), were harvested from University of Arkansas Research and Extension Centers at Stuttgart and Keiser, Ark. in 1999 and 2000. The samples were dried under three conditions, as shown in the experimental design (Fig. 1), for three durations aiming at removing 3.0, 4.5 and 6.0 percentage points MC (PPMC). Immediately after drying, the rice batch was divided into four sub-samples. One sample was immediately cooled by placing it in an equilibrium moisture content (EMC) chamber set at 21°C and 50% relative humidity, and left to gently dry to 12.5% MC. The other samples were tempered in a sealed bag for 80, 160, or 240 minutes at the temperature of the drying air before being taken out of the sealed bag to cool and then dry in the EMC chamber. The different drying durations created different MC gradients inside the kernel; subsequently, the different tempering durations allowed different MC gradient relaxation. This resulted in various levels of fissuring when the kernel was cooled and forced to undergo a transition from the "rubbery" to the "glassy" state. A control sample was put in the EMC chamber to gently dry to 12.5% MC, resulting in minimal fissuring and a high HRY. After storage for two months at 21°C, the rice was hulled with a laboratory huller. The immature and chalky kernels were separated and 400 brown rice kernels were randomly picked from each sample for the determination of the percentage of fissured kernels. One person using a light box visually observed the kernels for fissuring. Kernels were characterized as having surface cracks or internal fissures. Surface cracks were defined as fissures that appear on the outer surface of the kernel and internal fissures were defined as fissures that cut the kernel transversally or axially from one side to the other. After fissure counting, the kernels were returned to the dried sample to be milled, and HRY was determined using a FOSS Graincheck 310 image analyzer (Foss North America, Minneapolis, Minn.). A statistical analysis was not performed due to the fissure counting work being very time consuming, resulting in a limited number of samples.

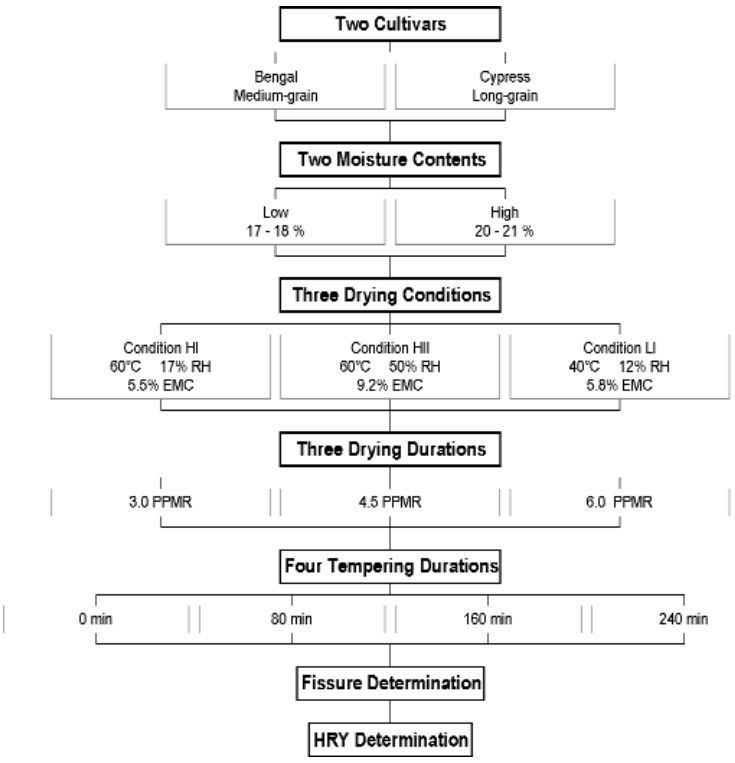

Fig. 1. Experimental design. RH is the relative humidity of the drying air. EMC is the equilibrium moisture content of the drying air. PPMR is the percentage points of moisture the drying durations were aiming to remove in one drying pass. HRY is head rice yield.

RESULTS AND DISCUSSION

Cnossen and Siebenmorgen (2000) concluded that if rice is tempered above the Tg line sufficiently long enough to reduce MC gradients, a state transition would not cause HRY reduction if irreversible damage has not yet occurred. Insufficient MC gradient reduction before a state transition will produce fissures and consequent HRY reduction. Fig. 2 shows the percentage of fissured kernels and the HRY versus tempering duration for Bengal (1999 harvest). A dramatically lower HRY was observed in the sample that was not tempered compared to a gently dried control sample. For the 3.0 PPMC drying duration, 80 minutes of tempering was sufficient to prevent fissuring and maintain an HRY equal to that of the control sample. The samples tempered for 160 and 240 minutes did not show a higher HRY. When removing 4.5 PPMC, 160 minutes of tempering was necessary to maintain an HRY near that of the control sample.

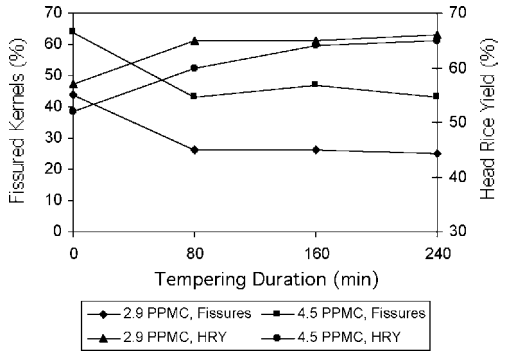

Fig. 2. Percent fissured kernels and head rice yield (HRY) versus tempering duration for Bengal rice harvested in 1999 and dried for two different durations (PPMC is the percentage points MC removed in one drying pass) with 60°C and 50% RH drying air. The percentage of fissured kernels and the HRY of the control sample were 24% and 65%, respectively. The harvest moisture content was 17.5%.

Tempering for 80 minutes reduced the number of fissured kernels. However, longer tempering did not further reduce the number of fissured kernels. Although the samples tempered for 160 and 240 minutes had an HRY equal to that of the control sample, these samples still had a higher number of fissured kernels (47 and 43%, respectively) than the control sample (24%). Thus, a large percentage of the fissured kernels did not break in the milling process.

The number of fissured kernels having internal fissures decreased with increasing tempering duration and increased with increasing moisture removal rates (Table 1). The two drying conditions above Tg (60°C) showed similar trends for percentage fissured kernels and HRY. The drying condition below Tg (40°C) showed a lower number of fissured kernels but a higher number or kernels having surface cracks. This condition did not cause HRY reduction compared to a gently dried control sample and tempering did not have any effect on HRY.

For all three drying conditions, surface cracks increased with increasing tempering duration (Table 1). These will normally appear as a result of drying the surface too severely, and the number of surface cracks would therefore be expected to increase with increasing drying duration; however the data do not reflect this.

For Cypress (1999 harvest), removing 4.0 PPMC did not cause any HRY reduction compared to the control sample, even with no tempering (Fig. 3). When removing 5.1 PPMC, the HRY did not further improve after 80 minutes of tempering. For both drying durations up to 160 minutes of tempering was required to minimize fissuring.

Cypress had much lower fissuring than Bengal. Due to a thinner kernel, Cypress is more resistant to fissuring. The number of kernels having surface cracks increased with increasing tempering duration and increasing drying duration in contrast to Bengal.

Table 1. Percent fissured kernels, percent kernels having surface cracks, and head rice yield for Bengal harvested in 1999 and dried under three drying air conditions, for three durations, and tempered for four durations. The harvest moisture content was 17.5%. The percentage of fissured kernels, the percentage of kernels having surface cracks, and the HRY of the control sample were 24, 4, and 65%, respectively.

Drying duration	Tempering duration (min)	Fissured kernels (%)	Surface cracks (%)	Head rice yield (%)
Drying air condition HII (60°C, 50% RH)				
2.9 PPMC[x]	0	44	1	57
	80	26	8	65
	160	26	9	65
	240	25	14	66
4.5 PPMC	0	64	6	52
	80	43	6	60
	160	47	4	64
	240	43	12	65
5.3 PPMC	0	69	3	32
	80	55	3	53
	160	53	6	57
	240	49	6	60
Drying air condition HI (60°C, 17% RH)				
3.5 PPMC	0	45	7	55
	80	31	9	65
	160	24	1	65
	240	28	6	66
4.6 PPMC	0	68	2	37
	80	47	6	60
	160	49	2	63
	240	40	8	64
5.8 PPMC	0	81	2	32
	80	65	0	47
	160	49	3	59
	240	57	5	59
Drying air condition LI (40°C, 12% RH)				
3.1 PPMC	0	21	11	65
	240	24	13	65
4.4 PPMC	0	31	19	65
	240	24	28	65

[x] PPMC is percentage points moisture content reduction in one drying pass.

Table 2. Percent fissured kernels, percent kernels having surface cracks, and head rice yield for Cypress harvested in 1999 and dried under three drying air conditions, for three durations, and tempered for four durations. The harvest moisture content was 18.0%. The percentage of fissured kernels, the percentage of kernels having surface cracks, and the HRY of the control sample were 3, 4, and 63%, respectively.

Drying duration	Tempering duration (min)	Fissured kernels (%)	Surface cracks (%)	Head rice yield (%)
Drying air condition HII (60°C, 50% RH)				
2.8 PPMC[x]	0	3	2	...63
	80	4	6	...62
	160	3	8	...63
	240	2	6	...63
3.9 PPMC	0	7	X[y]	...62
	80	4	X	...62
	160	3	X	...61
	240	2	X	...61
5.8 PPMC	0	8	11	...60
	80	6	19	...62
	160	7	18	...62
	240	6	24	...61
Drying air condition HI (60°C, 17% RH)				
2.5 PPMC	0	5	1	...63
	80	2	5	...63
	160	1	6	...63
	240	4	7	...63
4.0 PPMC	0	7	7	...61
	80	5	9	...63
	160	3	21	...62
	240	4	23	...63
5.1 PPMC	0	14	8	...54
	80	7	15	...61
	160	5	28	...61
	240	7	31	...62
Drying air condition LI (40°C, 12% RH)				
2.6 PPMC	0	2	4	...62
	240	3	5	...62
4.5 PPMC	0	2	23	...62
	240	3	38	...62

[x] PPMC is percentage points moisture content reduction in one drying pass. [y] Not measured.

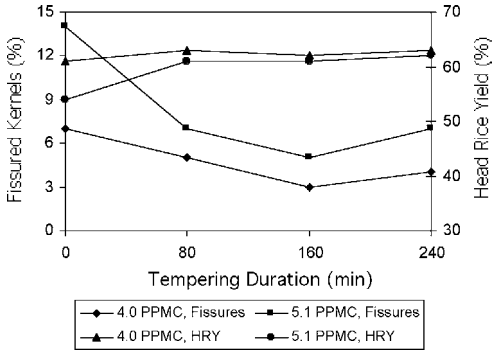

Fig. 3. Percent fissured kernels and head rice yield (HRY) versus tempering duration for Cypress rice harvested in 1999 and dried for two different durations (PPMC is the percentage points MC removed in one drying pass) with 60°C and 17% RH drying air. The percentage fissured kernels and the HRY of the control sample were 3% and 63%, respectively. The harvest moisture content was 18.0%.

However, no tempering was required to maintain an HRY equal to the control sample (Table 2).

For both Bengal and Cypress the 2000 samples showed similar trends as the 1999 samples (data not shown). For Cypress harvested in 2000, no tempering was required to maintain a high HRY when removing 4.7 PPMC, and only 80 minutes of tempering was necessary to maintain an HRY equivalent to the control sample when removing 6.1 PPMC.

The rice samples harvested in 2000 had a higher HMC than those harvested in 1999. The results showed that more moisture can be removed and shorter tempering durations are required to maintain a high HRY when harvesting at a higher MC. However, HMC did not seem to have an effect on the tempering duration required to minimize fissuring levels.

We conclude that kernels that fissure during drying and tempering do not necessarily break in the milling process and that the tempering durations required for preventing kernel fissuring might be longer than the tempering durations required for maintaining HRY.

ACKNOWLEDGMENTS

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Vasotocin receptor expression in the brain and pituitary gland during the ovulatory cycle of the fowl

Kelly R. Shaffer,[*] *Jorge A. Vizcarra,*[§] *and John Kirby*[¶]

ABSTRACT

Vasotocin receptors are members of seven transmembrane spanning G-protein associated receptors. Several isoforms have been recognized in mammals and birds. It has been shown that VT-1 expression occurs primarily in the brain while VT-2 expression occurs mainly in the pituitary. There is no current evidence to support that both VTR-1 and -2 are found in a single tissue. Our goal in this experiment was to see if VT-1 and VT-2 receptor mRNA expression varied in known sites of expression over the period of the ovulatory cycle of broiler breeder hens. In order to study potential changes in VT-1 and VT-2 expression, birds were sacrificed at 3 hour intervals over a 24 hour period. Blood samples were drawn. After cervical dislocation, the brain, pituitary, shell gland, and kidney were removed. Plasma was stored at -20ºC prior to determination of corticosterone levels by radioimmuno assays. Isolated mRNA from the brains and the pituitaries was transferred to nylon membranes for northern slot blot analysis. cDNA for VT-1 and VT-2 was used to make random primed cDNA probes. Corticosterone levels significantly increased at 9 hours post oviposition relative to all other times. Neither VT-1 or VT-2 expression showed any significant variation over the 24 hour cycle. Based on these results, we conclude that VT-1 and VT-2 steady state mRNA levels do not fluctuate dramatically over the ovulatory cycle of broiler breeder hens. Further work on membrane bound receptors and on circadian variations in membrane bound receptors in the brain and pituitary is currently underway of broiler breeder hens.

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INTRODUCTION

The effects of the circadian (~24 hr) rhythms on endocrine, reproductive, and immune system function in mammals and birds have been studied for many years. One circadian rhythm that is unique to certain birds, and laying hens in particular, is the daily production of a hard shelled egg. While the study of relationships among the various hormones, their receptors and the central regulation of hormone secretion has progressed dramatically over the past 10 to20 years, this research has lagged behind in avian species relative to mammals. Recently, we have been working with Larry Cornett, Department of Physiology, University of Arkansas Medical School and Dennis Baeyens, Department of Biology, University of Arkansas, Little Rock on the cloning and characterization of two new "novel" hormone receptors in the chicken. These receptors bind the small peptide hormone arginine vasotocin (AVT), an nonapeptide (nine amino acids), that is the avian homolog to mammalian arginine vasopressin (Hadley, 1996; Tan et al., 2000).

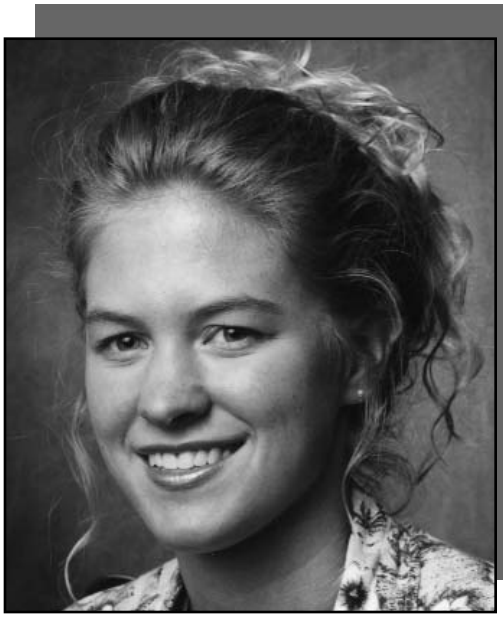
Arginine vasotocin has been shown to have anti-diuretic effects on the kidney (increased water retention, decreased urine production, and increased blood volume) and is the primary anti-diuretic hormone in chickens (Scanes, 2000). Further, AVT has additional functions that are similar to those associated with a second hormone, oxytocin, in mammals. These functions include the profound stimulation of uterine smooth-muscle contraction associated with oviposition (egg laying) and ejaculation (Rzasa, 1984). Finally, AVT has also been implicated in the stimulation of pituitary prolactin secretion, which regulates broody (mothering) behavior (El Halawani et al., 1992) and of adrenocorticotrophic hormone (ACTH), which stimulates the adrenal glands to produce corticosterone i.e., the primary glucocorticoid hormone associated with stress in birds (Castro et al., 1986).

The site of AVT synthesis is the neurohypophysis, the neuropeptide secreting portion of the posterior pituitary gland located just below the hypothalamus on the bottom surface of the brain. This hormone is then secreted into the circulation via a diffuse capillary bed where it travels throughout the body or, alternatively, AVT can be secreted into the intracellular spaces associated with the adenohypophysis (anterior pituitary gland) to rapidly affect the production of pituitary hormones such as prolactin, ACTH, luteinizing hormone (LH), and follicle stimulating hormone (FSH). The receptors for AVT, VT-1 (Tan et al., 2000), and VT-2 (Baeyens, Cornett, Vizcarra, and Kirby, unpublished)

Meet the Student-Author

I was born in Fayetteville and graduated from Rogers High School, which is where my family currently lives. I am a sophomore at the University of Arkansas. I am a member of Gamma Beta Phi and work with the Baptist Student Union, including being a part of their housing ministry. I have been awarded a University Scholarship, a Poultry Science Scholarship, and a Tyson Memorial Scholarship. My freshman year, I was on the Dean's List and in Spring 2001 made the Chancellor's list with a 4.0 grade point average.

I hope to pursue a career in medicine, either as a nurse or a doctor. My love for medicine stems from a desire to help people. My goal is to become a missionary overseas where there is a desperate need for more people in the medical field. I feel that helping people with their health gives me a chance to share with them the love of God. It is through medicine that I feel I will best be able to use the skills that God has given me and express the love for people that I have.



Kelly R. Shaffer

have been cloned, sequenced, and expressed in vitro. In preliminary work it has been shown that the VT-1 receptor is expressed in the brain and oviduct and that the VT-2 receptor is expressed in the pituitary of hens (Baeyens, Cornett, Vizcarra and Kirby, unpublished). However, the temporal, spatial, and functional differences between these receptors have not been determined.

Our objective in this research was to characterize the circadian, or ovulatory cycle, variation in tissue specific mRNA expression for VT1 and 2 in the context of fluctuations in plasma corticosterone, LH and FSH.

MATERIALS AND METHODS

Sexually mature broiler breeder hens (Cobb 500) were placed in individual cages on the U of A Poultry Farm and maintained on a daily schedule of 16 hours of light and 8 hours of dark with lights on at 0600 hours and lights off at 2200 hours. The time of oviposition was recorded for each hen in the experiment, and all times are expressed as hours relative to the recorded oviposition (e.g., +3 hours, 3 hours after an egg was laid).

Experimental. At three-hour intervals, beginning at time 0 (oviposition) and ending 21 hours later, four hens were selected per time point and bled by venipuncture with plasma collected and frozen for subsequent radioimmunoassay (RIA) determination of corticosterone, FSH, and LH. After blood sampling, hens were killed by cervical dislocation and the brain (principally hypothalamus), pituitary, and kidney were removed and frozen in liquid nitrogen for total RNA isolations.

RNA Analysis. Following the completion of all tissue collections, total RNA was isolated, separated by electrophoresis and transferred, or in the case of pituitaries, slot-blotted, to a nylon membrane using standard procedures (Sambrook, et al., 1989). Filters were then sequentially probed with P32- labeled, randomly primed cDNA probes for VT-1, VT-2, and a 28S ribosomal RNA probe. Relative expression of each RNA was then quantified using a Typhoon phosphor imaging system. Relative levels for VT-1 and VT-2 expression were expressed as a percentage of the 28S ribosomal RNA control. Relative RNA expression levels were analyzed using the General Linear Models procedure in SAS.

Radioimmunoassays. Luteinizing Hormone (LH) concentrations were measured by RIA using reagents provided by the US Department of Agriculture-Agricultural Research Service Animal Hormone Program. Concentrations of Follicle Stimulating Hormone (FSH) (Krishnan et al., 1993) in plasma (150 _l) and pituitary gland extracts were quantified in duplicate by RIA using reagents provided by the USDA/ARS. Concentrations of corticosterone in the plasma were quantified by RIA similar to that previously described (Proudman and Opel, 1989) with reagents provided by Dr. John Proudman, USDA-ARS/GGPL, Beltsville, Md. Changes in plasma and pituitary hormone concentrations were analyzed using the General Linear Models procedure in SAS 2000.

RESULTS AND DISCUSSION

Northern analysis revealed expression of mRNA for VT-1 in the brain, for VT-2 in the pituitary, and expression of neither VT-1 nor -2 in the kidney. Upon inspection of these steady state mRNA levels, no circadian or ovulatory cycle state variations in either VT-1 nor VT-2 receptor steady state levels were detected. These results suggest that if variations in AVT receptor levels are associated with circadian or ovulatory cycle changes, these changes are independent of receptor mRNA level. Alternatively, as steady state mRNA levels can not identify changes in coupled transcription rate, translation efficiency, or mRNA stability, a different assay may have provided better insight. Furthermore, until an effective receptor binding assay is developed for the VT-2 receptor, it will be difficult to assess receptor protein levels or function.

While the VT-1 and VT-2 mRNA levels failed to show any significant variation in expression over the 24-hour sampling period, plasma levels of corticosterone showed a clear circadian pattern of variation (Fig. 1). The pattern shown in Fig. 1 is the observed peak in plasma corticosterone concentrations approximately 9 hours after oviposition (and about 12 hours after lights on). This is quite different from the pattern observed in humans, with the highest observed levels of glucocorticoids occurring within 1 hour of exposure to light (reviewed in Griffen and Ojeda, 1988). This peak in plasma corticosterone occurred in the absence of any observed change in AVT receptor expression; it coincided with the nadir in plasma FSH and precedes that of plasma LH by one sampling period. As the inhibitory effects of elevated corticosterone levels are

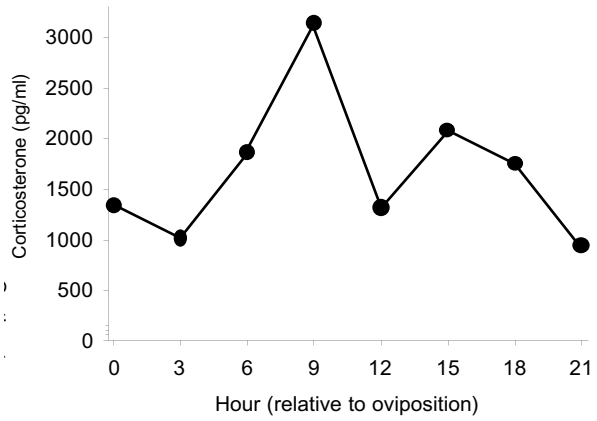


Fig. 1. Plasma concentrations of corticosterone in laying broiler breeder hens over an ovulatory cycle. The circadian rhythm of corticosterone is evidenced by the significant ($p<.05$) elevation in plasma concentration observed at 9 hours post oviposition. Hens (4 per time point) were selected and killed at three-hour intervals relative to oviposition; all hens represented would have produced an egg on the day following sampling as well. Blood samples were collected within 2 minutes of capture, and corticosterone levels were determined by radioimmunoassay.

well documented, this observation fits the previously described experimental results (Griffen and Ojeda, 1988; Hadley, 1996).

As documented previously by (Krishnan et al., 1993), plasma FSH levels vary by about two-fold over the ovulatory cycle (Fig. 2), with the peak levels of

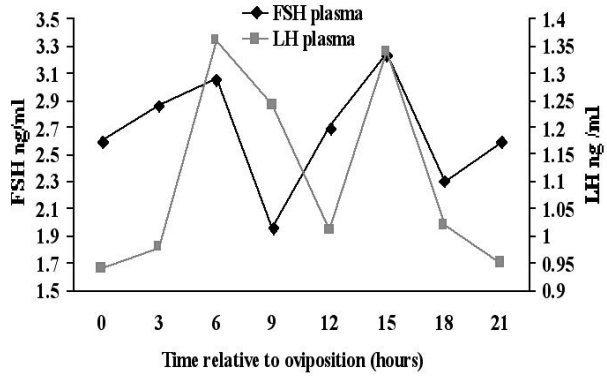


Fig. 2. Plasma concentrations of FSH (diamonds) and LH (squares) in laying broiler breeder hens over an ovulatory cycle. The ovulatory rhythms of FSH and LH are evidenced by the significant ($p<.05$) elevation, decline and elevation again in plasma concentrations observed between six and fifteen hours post-oviposition. Hens (4 per time point) were selected and killed at three-hour intervals relative to oviposition; all hens represented would have produced an egg on the day following sampling as well. Blood samples were collected within 2 minutes of capture, with LH and FSH levels determined by homologous radioimmunoassay.

plasma FSH occurring during the period of follicular recruitment (<9 hrs post oviposition) and immediately before the peak of the preovulatory LH surge at 16-20 hours post oviposition. Observed LH levels were somewhat lower in this experiment than has been described for the ovulatory cycle in leghorn chickens (Johnson and van Tienhoven, 1980); whether this is due to the use of a different assay, standards, or fundamental differences between leghorns and broiler breeders cannot be determined from this data. The sampling interval used here, 3 hours, more than likely missed the peak of the preovulatory LH surge (Fig. 2), however, the observed significant increase in LH suggests that the hens would have ovulated an egg on the morning of sacrifice. These daily variations in plasma LH and FSH concentrations have been shown to be critical in the regulation of the ovulatory cycle of the hen (Johnson, 2000).

Pituitary contents of FSH and LH (ng/mg tissue) showed considerable variation over the ovulatory cycle as well (Fig. 3). These variations in pituitary contents

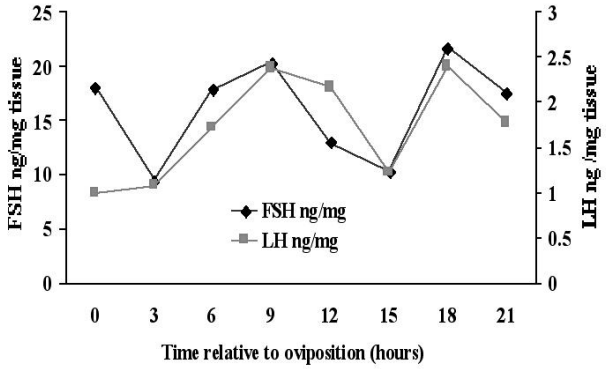


Fig. 3. Pituitary contents of FSH (diamonds) and LH (squares) in laying broiler breeder hens over an ovulatory cycle. The ovulatory rhythms of FSH and LH are evidenced by the significant ($p<.05$) decline, elevation and decline again in pituitary contents observed between three and fifteen hours post oviposition. Hens (4 per time point) were selected and killed at three-hour intervals relative to oviposition; all hens represented would have produced an egg on the day following sampling as well. Pituitaries were removed immediately following death, frozen and homogenized, with LH and FSH levels subsequently determined by homologous radioimmunoassay.

are most likely associated with either increased synthesis of hormone, decreased rate of secretion or turnover, or some combination thereof. We have observed that variations in both plasma and pituitary concentrations of both hormones follow a similar pattern, when we

advanced pituitary contents by 3 hours, we saw that the data coincide. The changes observed in pituitary hormone contents were preceded by changes in plasma hormone levels by about 3 hours (data not shown). These results suggest that rates of secretion, as suggested by plasma hormone concentrations, account for much of the variation observed in pituitary contents. That is, increased secretion leads to higher plasma concentration and reduced pituitary content. As these samples were collected from different individuals at each time point, these data provide insight into the robustness of the daily ovulatory cycle.

In summary, we were unable to demonstrate any circadian variation in the pattern of AVT-receptor mRNA expression in either the brain or pituitary at the gross level. Whether or not finer variations in mRNA expression occur or whether variations in functional receptor levels vary over the ovulatory cycle cannot be determined from this study. However, the hens studied clearly demonstrated daily rhythms of corticosterone, FSH, and LH. An interesting relationship between pituitary LH and FSH contents and plasma levels of these hormones was observed, with pituitary levels changing approximately 3 hours after observed changes in plasma levels. Further work to characterize changes in AVT-receptor expression changes will be needed to more completely answer this question.

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Development of the interactive broiler income spreadsheet

Tara Shofner[*] *and H.L. Goodwin, Jr.*[§]

ABSTRACT

The poultry industry has experienced unprecedented increased efficiencies since 1960 in large part due to vertical integration facilitated by production contracts between growers and integrators. As growers seek information about contract production they need to be well informed about all aspects of the process, especially potential income. Recent poultry grower complaints have surfaced as a result of incorrect expense and revenue expectations. The Interactive Broiler Income Spreadsheet (IBIS) is being developed to enable current and prospective poultry producers to better estimate income. IBIS, an unbiased Excel™ spreadsheet tool to assist in decision making regarding broiler production profitability, uses actual grower expense and revenue information or, alternatively, grower-panel default data to assess income under various grower-specified production, expense, and price scenarios. Poultry integrator grower service personnel, lenders, and Cooperative Extension professionals will utilize IBIS to assist growers in operational planning and risk tolerance identification in varying economic situations. Growers may also gauge effects of capital improvements, equipment upgrades, chick placements, and time between flocks on income. Development of IBIS is continuing with collection of additional data and revision of procedures based upon results of field testing.

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INTRODUCTION

The poultry industry has evolved from chickens roaming in the backyard to highly specialized operations that produce a total of 900 billion birds a year for meat. The poultry industry has experienced unprecedented success in production and marketing efficiencies. One of the reasons this success has been a direct result of the use of contracts between the grower and the integrator. Contracts have worked very well for a number of years; however, recently there have been many complaints from poultry growers (Banker, et al. 1997). Part of the problem is a result of poultry growers' incorrect expectations about projected expenses and revenues. There is no publicly available data to examine grower returns; therefore, it is nearly impossible to determine the overall financial situation of poultry growers (Rogers, 1992). For the most part, growers make their business decisions regarding the feasibility of new or expanded poultry farms based upon information provided by integrators or from an informal network of other poultry growers in their area.

As potential growers seek information about contract production, they need to be well informed about all aspects of the process, especially the potential income. Poultry production is capital-intensive. The estimated investment for a fully equipped poultry farm in 1996 was $100,800 for a 42 ft. x 500 ft. house, with most farms having at least two houses (Vukina, 1998). Even though poultry farmers invest 50% of the capital required for producing the final products for the industry, over 71% of contract growers earn a net income below the poverty level from their poultry operations (Krebs, 1999). A major risk that the grower faces is the capital cost of the land, and the degree of the asset fixity for the buildings and equipment, since they have no good alternative use (Rogers, 1990).

Many integrators give the growers only oral information about the profits that they will receive under the contract (G. Harral, personal communication). This may be because the integrator does not have complete information to give a potential grower. One major problem is that individual poultry operations may not generate the initial profits anticipated based upon information obtained from the integrator or the informal grower network. Even if profits are in line with projections initially, they may decline in subsequent years, making it necessary for the farmer to seek other

Meet the Student-Author

Tara Shofner

I graduated in May 2001 with a degree in agricultural business, and I am now working on a master's degree in agricultural economics. Dr. H.L. Goodwin, my undergraduate faculty sponsor for the broiler production spreadsheet project, is now my academic advisor for my graduate studies.

I am a native of Elkins and a 1997 Elkins High School graduate. I am one of several UA graduates in my family with degrees in agriculture or home economics (now human environmental sciences). Other UA alumni include my father and his parents and an aunt and uncle. My brother, Travis, is a senior majoring in turfgrass management at the UA.

The opportunity to conduct research as an undergraduate student helped me decide to pursue graduate study. My research experience was one reason for my selection as the UA student representative at the Princeton Today International Conference in New York City. I also won two research presentation contests, one each sponsored by the American Agricultural Economics Association and the Arkansas chapter of Gamma Sigma Delta.

I was a summer 2001 intern with the Arkansas Farm Bureau Federation in Little Rock, which helped prepare me for my career goal of working in agricultural marketing.

income opportunities (H.L. Goodwin, unpublished data). In late 1999, the Arkansas Farm Bureau Federation (AFBF) asked the University of Arkansas to conduct a survey of 1300 of its members that were poultry growers (Goodwin, in press 2001). These growers were asked to rank their satisfaction with various aspects of the poultry business. Many acknowledged discontentment with the financial returns in their poultry operations. For example, of the 288 respondents, 56% of growers expressed some degree of dissatisfaction with the income that they receive from their poultry operations. Sixty-seven percent stated that they are not getting a fair return on their investment. Respondents also contended that they are unhappy about the communication between themselves and their integrator. Eighty-four percent of respondents agreed with the statement, "My company should provide educational programs to help producers better estimate income and expenses." In response to the statement "Communication between growers and companies is adequate," 53% of respondents disagreed. In the free response section, one grower stated, "There is not enough information for potential growers," and almost 45% of growers surveyed by the AFBF survey said there is not adequate problem-solving information available to them.

Many producers find it necessary to have off-farm income. Over 47% of respondents of the AFBF survey revealed that their spouse had either part-time or full-time off-farm employment. There simply may not be adequate net income from the average 3.4 house poultry operation to support a household. This is particularly the case if substantial debt service on the operation exists.

Problem Statement. There are several reasons why profitability from broiler operations is so difficult to forecast. First, it is still nearly impossible to effectively determine revenue for poultry growers because of the grower pay system used throughout the industry. The grower payment amount may not actually reflect the grower's performance compared to an average grower, but rather to the other growers who sell in the same weekly pool. The pool takes all the producers who sell in the week and ranks them by their cost of production (Doye, 1996). The middle grower receives the base pay amount only. If the growers' cost of production is lower than the middle, they receive the base pay plus a premium proportional to their ranking. If their costs are above the middle, they are penalized and receive a discounted base pay. Therefore, the actual amount that the grower receives in base pay and bonuses depends on performance of other growers that sell in the same week.

Secondly, estimating income may be difficult because of varying poultry house size (Doye, et al., 1996). While most new poultry houses are built on a standard house size, many older houses were not built to any standard size. Variable dimensions of older houses can also lead to difficulty in estimating profitability. Many potential growers are faced with trying to estimate revenues and expenses from a standard estimate sheet provided by the integrators.

Finally, many potential poultry farm sellers are not usually willing to supply all of their past records to be evaluated before the sale of their farm. Potential growers may find it very difficult to get an accurate approximation of the farm's past performance. And, as alluded to previously, integrators do not have accurate records for growers possibly due to the lack of communication and because they view the growers as independent contractors for grower services.

Budgets play an important role in planning for any new investment. The two types of budgets of particular interest to poultry farmers are capital investment budgets and enterprise budgets. Budgets aid in the systematic evaluation of alternative plans by putting the plans "on paper" to determine which will maximize profits (Kay and Edwards, 1999). They can be helpful in planning, implementation, and control of any farm business.

Major capital purchases should be carefully analyzed and planned to make certain they fit into the long-term operation of the business. Given the large amount of capital that poultry farms must borrow, capital budgeting is one of the most important financial management tools available to producers (Beierlein et al., 1995). For many poultry producers, capital budgeting does not end after the initial investment of houses and equipment, but continues with the investment in company-required upgrades. As new technology is introduced, many poultry operations are obligated by their contracts to upgrade or replace existing equipment.

Enterprise budgets organize projected income, expenses, and profit of a single enterprise (Kay and Edwards, 1999). These budgets may be published by the Cooperative Extension Service or the poultry companies such as Tyson's, Perdue, or Gold Kist. Enterprise

budgets are very general and are a good starting place for prospective growers to begin their research into poultry farming. However, they may use assumptions that can skew projections of profitability. Most of these budgets do not break down the costs into enough detail. Growers are also concerned about the hidden expenses that are not explicitly described on these enterprise budgets or by the integrator (Cunningham, 1995). Each poultry operation is unique, and many of these budgets do not reflect different factors such as assorted house sizes, litter as an expense or revenue. They may also disregard the extreme discrepancy between utility expenses due to variable natural gas, propane and electricity rates and the use of wells versus municipally-treated water.

Objectives. The overall objective of this project was to help prospective and current poultry producers to better estimate profits by developing the Interactive Broiler Income Spreadsheet (IBIS). IBIS is an unbiased tool using Excel™ software that will be made available to existing and prospective growers to use as they make decisions regarding the current and potential profitability of raising chickens. Specifically, it will:

1. Allow growers to more precisely estimate revenues and expenses;
2. Allow growers to calculate the feasibility of new investments;
3. Allow growers to easily change any of the factors that will influence estimates of revenues and expenses to reflect current weather, price, interest, or regulatory conditions.

MATERIALS AND METHODS

The first step in this project was to develop a data collection sheet. The information collected from this sheet was used as the default information. It was important to have default data, especially for potential growers who have no records of their own. This collection sheet was also used as a foundation for the spreadsheet. The data collection sheet gathered information about all areas of production expenses and revenues for each of 4 years. The data collection sheet was modified several times, as it became apparent that important information was excluded. One of the most important steps of this project was to accurately reflect all of the expenses that are incurred by poultry growers. Many of the expenses were broken down into usage amount and price per unit instead of simply total cost to be more precise.

After the data collection sheet was developed, grower participation was needed. The data collection sheets collected information from contract growers from the four largest poultry integrators in northwest Arkansas. Those companies are George's, Peterson Farms, Simmon's Industries, and Tyson Foods. The companies approved the participation of at least four contract growers from their companies. These growers were selected from the top one-third of each production complex based on their past performance and record-keeping practices. All information collected was confidential and no names of the growers or integrators were requested on the data that was collected.

Data were collected though personal contact. Each of the four growers were mailed a data collection sheet with a cover letter explaining the purpose of the research. Each letter was followed up by a telephone call to answer questions. In addition, farm visits were made utilizing the same data collection sheets as previously mailed. This additional step proved to be most successful. Many of the growers were not easily reached by phone and felt too busy to sit down and answer numerous questions about their farm; however, all the growers were more than happy to answer questions during the visit. To date, information from eight growers has been obtained, verified, and analyzed, and four others have agreed to personal visits. In addition, all grower information will be averaged before this panel data will be used as default values for the various cost and income components of IBIS.

RESULTS AND DISCUSSION

Interactive Broiler Income Spreadsheet Development. IBIS was developed using Excel™ software. A sample of IBIS is located in Table 1. The sample data presented was from one of the farms included in the data collection phase. The sensitivity of the program can not be adequately observed in the sample; however, the sample does provide a look at the inputs and outputs of the formulas. IBIS is divided into two parts: assumptions and budget analysis. The assumption section is divided into house dimensions, estimated income, estimated expenses, and loan information. The budget analysis section takes the information from the assumptions and computes profits.

The assumption section begins with the "House Dimensions" segment that totals the number of houses

and computes the total square footage of the poultry houses. Since most houses are built in a few standard sizes, the sizes 40 ft x 400 ft, 40 ft x 200 ft, 32 ft x 400 ft, and 42 ft x 500 ft are formatted so that the user only has to enter the number of each of those sized houses they operate. However, there are many poultry houses that do not fit into one of these four typical sizes. IBIS is designed so users may enter up to three unique house sizes along with the number of houses of that particular size. The total square footage is used in the default formulas to figure the net cash returns on a square foot basis. This allows users to compare returns on different size operations.

Next in the assumption section, users are asked to fill in cells with their information or utilizing the provided default numbers. After the user completes the "House Dimensions" section many of the default values automatically adjust based on the number of houses and total square footage of their operation. Many of the default values have formulas that allow for a more accurate value based on either the number of houses, number of chicks, or total square footage. Many current growers, however, will have their own records that more precisely reflect their operation.

The income section separates all areas of possible income-generating activities. Many poultry producers have other enterprises that supply income. Some of the farmers who participated in data collection had cattle, sheep, goats, and/or hay operations. IBIS, however, only includes the income that is directly derived from poultry operations. Default information is provided for almost every category except gas and utility allowances and the average bonus amounts. These three items vary tremendously by company, geographic location, and individual grower preferences. Use of any default amount could be very misleading; therefore, the individual integrator or producer can better estimate these values.

The expense section is divided into variable and fixed expenses. Usage amount and price per unit divide many of the variable expenses. The fixed expenses include taxes, insurance, depreciation, and opportunity costs. Many of the fixed expenses do not have default values because they are things such as initial investment amount on houses and equipment, interest rates on loans, and cost of land. These values will vary by user. Below the "Estimated Expenses" section is the "Loan Information" section. The section asks for basic loan information that will be used in the budget analysis below. There are three areas for loan information: house loans, equipment loans, and upgrade loans. Many users may not utilize all three areas. Some may have a combined house and equipment loan. Also, current producers may only need to compute the payments on an upgrade if that is what they are considering.

Also included to the right of the assumption section are question and answer prompts. These help clarify the particular information being asked for and help to answer question that may arise from various growers in actual farm situations. In the IBIS example that is attached, only a select number of prompts are shown. For instance, several of the questions address the different uses of litter. Litter is included in both the revenue and expense sections. This is because litter can be of value to growers if spread on their own farms or if sold to another farmer to spread. If growers use the litter on their own farms it is a credit, and if sold, it is a cash revenue. However, litter can also be an expense if the grower must pay someone for clean-out and disposal. This would be the case if the grower either did not have the land area or the desire to spread the litter. Other prompt questions cover issues such as company utility allowances, dead bird disposal cost, and water supply.

The "Budget Analysis" section uses the information gathered in the "Assumptions" section and computes total operating revenue, total operating expenses, total fixed expenses, total expenses, net farm income, net farm income per square foot, net cash returns, and net cash returns per square foot. The budget analysis includes both budget value and cash value. The net cash income is computed by converting the revenue information entered into a pay formula of:

Chicks per flock x Flocks per year x (100-Percent mortality)/100 x Average pounds per finished bird x Cents per pound (contract base)/100

The other poultry related, income-generating activities then add to the pay formula to get the total operating revenue. Those include litter revenue, gas allowances, utility allowances, and performance bonuses. Total operating expenses are then subtracted from total operating revenues to get net cash returns. Net cash returns per square foot are simply net cash returns divided by the total square footage computed in the assumption section. Net farm income is computed by taking the the total budget value expenses from the total operating revenues.

IBIS Verification. Continual verification of the effec-

tiveness and accuracy of the IBIS software is underway. Poultry integrators in northwest Arkansas were consulted about the feasibility of this project and were instrumental in collecting data for IBIS. Current poultry producers gave advice on the areas of revenues and expenses that should be incorporated, including many hidden expenses that were not in any of the published budgets. With the completion of the IBIS program, verification will continue to take place. A panel consisting of four lenders is being asked to compare IBIS results with their records. Also, trial runs are being conducted through field tests with current University of Arkansas poultry science students and with the guidance of Cooperative Extension Service specialists. After verification is complete, IBIS will be released to the public and monitored as the poultry industry changes to keep the program up-to-date and functional.

Application of Results. IBIS will be available to producers though the poultry integrators, area lenders, the Cooperative Extension offices, and a University of Arkansas website. IBIS will be primarily used by the poultry integrators as a decision-making tool for potential growers. By having this interactive software, they will be able to play "what if" games to identify their risk tolerance to varying income and expense levels. IBIS can be an effective training tool for service personnel and can be used to demonstrate to growers the income effects of management decisions. In addition, growers will have the capacity to gauge the effects of capital improvements/equipment upgrades and chicken placements per year.

As useful as IBIS can be, even the best farm management programs are of no use if producers do not have the skills, technology, or desire to use them. In the AFBF survey, while 60% of respondents used a computer in their farm operation, only 36% of those with a computer used a spreadsheet program. Many farmers do not see the need to implement computer technology in their daily operations. Even those who do use computers often do not have the knowledge to use this technology to their full advantage. It is also important to remember that even the best budget planning cannot take the place of good management. IBIS is simply a tool to help management be more effective.

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Table 1. Interactive Broiler Income Spreadsheet

(This example is for the first year only of broiler production in this operation)

I. Assumptions Section

A. Initial Questions

1. Do you have tunnel ventilation? If **YES** enter 1, if **NO** enter 0	0
2. Do you have cool cell? If **YES** enter 1, if **NO** enter 0	0
3. Do you have foggers? If **YES** enter 1, if **NO** enter 0	0

B. House Dimensions

		Enter house # here	
Dimensions of houses:	40x400	3	48000
	40x200	0	0
	32x400	0	0
	42x500	0	0
Enter other size houses HERE		0	0
Enter: **=30*400**, NOT 30x400		0	0
		0	0
	Number of Houses	**3**	
	Total Square Footage		**48000**

After enter your house size information some of the default values will automatically adjust
Please enter your own information if it is more accurate than the default values
Some default values cannot be estimated and "none" appears in the cell
If you have questions, please click on Questions??? for further clarification

C. Estimated Revenues	**Default**	**Your farm**	
1. Chicks per flock	68570	60000	Questions???*
2. Flocks per year	5.5	5.5	Questions???
3. Percent mortality	4.5	4.4	Questions???
4. Ave lbs./finished birds	5.4	5.5	Questions???
5. Cents/lb. contract base	4.5	4.5	Questions???
6. Annual tons of litter	360	360	Questions???
7. Price per ton of litter	6	15	Questions???
8. Annual gas allowance	none	5112	Questions???
9. Annual utility allowance	none	0	Questions???
10. Annual average bonuses	none	5445	Questions???
D. Estimated Expenses			
Variable Expenses			
1. Annual trailer loads of bedding	3	3	Questions???
2. Price per trailer load of bedding	975	975	Questions???
3. Annual number of clean out loads	36	36	Questions???
4. Price per clean out load	30	30	Questions???
5. Annual number of cake out loads	30	30	Questions???
6. Price per cake out load	30	30	Questions???
7. Annual number of propane gallons	7398	7398	Questions???
8. Price per propane gallon	0.64	0.7	Questions???
9. Annual number cubit feet natual gas	6850	6850	Questions???
10. Price per foot natual gas	none		Questions???
11. Annual number of kilowatt hours	86400	86000	Questions???
12. Price per kilowatt hour	rates in Q/A	0.057	Questions???
13. Annual gallons of drinking water	743500	743500	Questions???
14. Annual gallons of water for other uses	0	7590	Questions???
15. Price per 1000 gallons of water	rates in Q/A	2.75	Questions???
16. Annual repair costs on facilities	none	500	Questions???
17. Annual cleaning supplies cost	none	500	Questions???
18. Annual pest control costs	none	1000	Questions???
19. Annual dead bird costs	none	2500	Questions???
20. Annual hours of paid labor	none	200	Questions???
21. Hourly wage, paid labor	6	6	Questions???
22. Annual paid labor for services	none	1500	Questions???
23. Annual misc. expenses	none	1200	Questions???
Fixed Expenses			
1. Annual insurance cost	2925	1875	Questions???
2. Annual property taxes	none	3000	Questions???

3. Annual land charge	none	4000	Questions???
4. Initial house investment (exclude house equip.)	none	330000	Questions???
5. Salvage value on house	none	20000	Questions???
6. Years in house life	30	30	Questions???
7. Initial house equipment investment	none	60000	Questions???
8. Salvage value on equipment	none	5000	Questions???
9. Years in equipment life	15	15	Questions???

E. Loan Information

Note: If the loan is not applicable to your farm, enter 0 on the "Amount borrowed" line of that loan.

House Loan

1. Interest rate on house loan	0.09	Questions???
2. Number of years in loan	15	Questions???
3. Number of payments per year	4	Questions???
4. Amount borrowed on houses	300000	Questions???

Original Equipment Loan

5. Interest rate on equipment loan	0.09	Questions???
6. Number of years in loan	15	Questions???
7. Number of payments per year	4	Questions???
8. Amount borrowed on equipment	15000	Questions???

Upgrade Equipment Loan

9. Interest rate on upgrade loan	0.09	Questions???
10. Number of years in loan	10	Questions???
11. Number of payments per year	4	Questions???
12. Amount borrowed on upgrade	0	Questions???

II. Budget Analysis Section	**Budget value**	**Cash value**
Poultry contract	78081	78081
Litter revenue	5400	5400
Allowances	5112	5112
Bonuses	5445	5445
Total Operating Revenue	**94038**	**94038**
Litter removal	4905	4905
Utilities	12146	12146
Repairs	7590	7590
Maintenance	4500	4500
Labor cost	2700	2700
Misc. expenses	1200	1200
Total Operating Expenses	**33041**	**33041**
Insurance	1875	1875
Property taxes	3000	3000
Annual land charge	4000	0
Depreciation	14000	0
House payment	27154	27154
Equip. payment	1358	1358
Upgrade payment	0	0
Total Fixed Expenses	**51387**	**33387**
Total Expenses	**84428**	**66428**
Net Farm Income	**9610**	
Net Farm Income Per Sq. Ft.	**0.200**	
Net Cash Returns	**27610**	
Net Cash Returns Per Sq. Ft.	**0.575**	

*** Hot Button Prompts for "Questions???" Regarding Poultry Litter**

Q-What if I don't have a total clean out each year?
A-Allow 12 ton per decade.
Q-What if someone cleans out my houses for only the litter?
A-Enter 0.
Q-What if someone cleans out my houses and pays me?
A-Enter 0.
Q-What if someone cleans out my houses and spreads the litter on my farm?
A-Enter 0.
Q-What if someone cleans out my houses and pays me?
A-Enter 0, that income will be credited above.

Lagging behind: Fayetteville's historic architecture

Jennifer Taylor[*] *and Jennifer Webb* [§]

ABSTRACT

Architecture is a reflection of what is happening in the larger cultural, economic, and artistic scene. Therefore, understanding regional variations in trend adoption is significant to understanding the relationship of Fayetteville, Ark., to the larger national context. Local architecture is a reflection of the citizens of Fayetteville as consumers of popular culture. Simultaneous adoption theory was used as the framework of this study. The project objectives were to 1) document significant architectural styles within designated historical districts and nearby areas, and 2) compare local stylistic trends with national trends to determine fit. Findings indicate that Fayetteville lagged behind the national trend in architectural styles during its early years but that increased transportation connections and the establishment of the University of Arkansas may have helped to move the area into the mainstream.

[*] Jennifer Taylor is majoring in interior design in the School of Human Environmental Sciences.

[§] Jennifer Webb, faculty sponsor, is an assistant professor in the School of Human Environmental Sciences.

INTRODUCTION

Architectural trends are a reflection of the relationship between geographical regions and the nation at large. Rifkind (1980) states "buildings, streets and landscape configurations speak of history and culture, art and technology, time and events." McAlester and McAlester (2000) further explain that homes are reflections of fashion. They explain that "most surviving American houses are not folk houses but are styled; that is, they were built with at least some attempt at being fashionable. As such, they show the influence of shapes, materials, detailing, or other features that make up an architectural style that was currently in vogue."

Simultaneous adoption theory explains the rise and fall of fashion styles (Sproles, 1985) and can be applied to a variety of creative fields in which the public consumes the product. The theory explains that innovators and forward thinkers initially adopt a trend or fashion during its introductory stage. These styles are introduced to the public by these innovators, and the subsequent stage, acceptance, results in mass market consumption of the style at its peak of popularity. The final stage, regression, is the decline of the style when it is adopted by fashion isolates (through lack of awareness) or fashion laggards (due to social pressure or economics). The graphic model generated by Sproles (1985) indicates that a bell-shaped curve accurately represents the introduction, acceptance, and regression of a particular style during its life-span. Adoption theory is appropriate not only to fashion but also to other stylistic trends such as art, architecture, and design. The suggestion that trends have a definable life span provides a foundation from which a comparison of regional to national trends can be made.

Architecture is a reflection of what is happening in

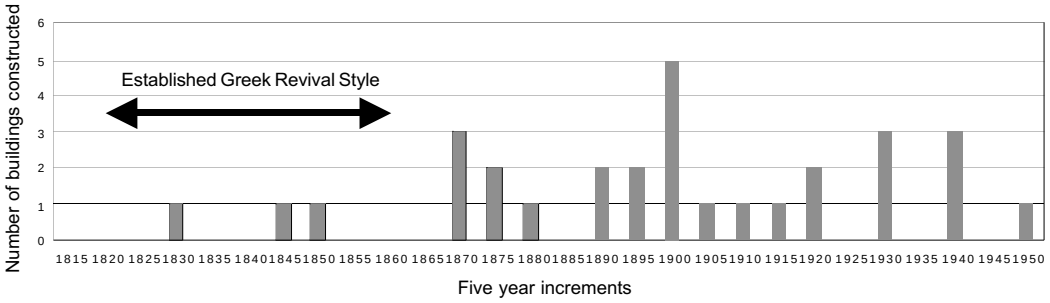
Meet the Student-Author

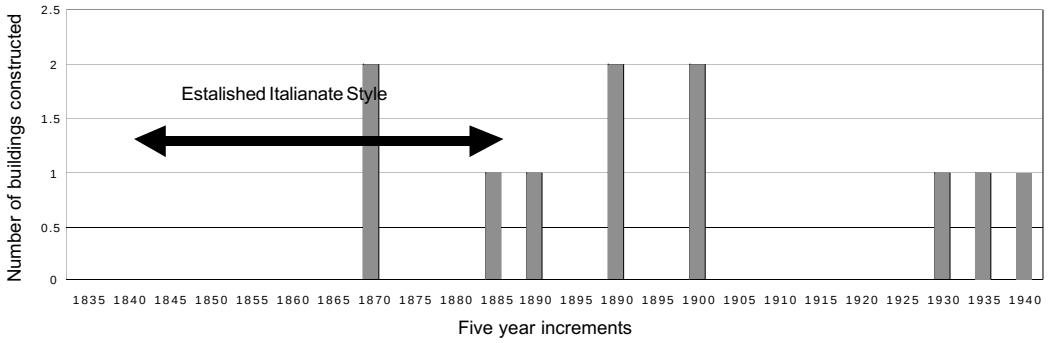
After graduating from Springdale High School in 1990, I attended the University of Central Arkansas in Conway where I received a BSE in history. While I was at UCA I completed an internship at the Old State House Museum where I provided tours of the facilities to school groups and the general public, as well as assisting the director of docents in developing educational programs and summer classes. I also worked part-time for two semesters at the Arkansas Museum of Science and History where I presented interactive programs that included story telling, role playing, and the handling of artifacts and animals. As a result I have a strong background in Arkansas and Civil War history. After taking a few years off, I enrolled in the interior design program at the U of A and plan to work in the field of historic preservation upon graduation. I attended the 2001 session of the Nineteenth-Century Studies' Summer School in Newport, R.I., sponsored by the Victorian Society in America.

I chose to do this research project because of my combined interests in history, architecture, and decorative arts. This was a great opportunity for me to become more familiar with American architectural styles of the 19th and early 20th centuries. I also made some very good contacts in the historic preservation field and learned a lot of interesting history about the city of Fayetteville.

Many thanks to the people at the Arkansas Historic Preservation Program in Little Rock and to my advisor, Dr. Jennifer Webb, for her assistance throughout this project.

Jennifer Taylor





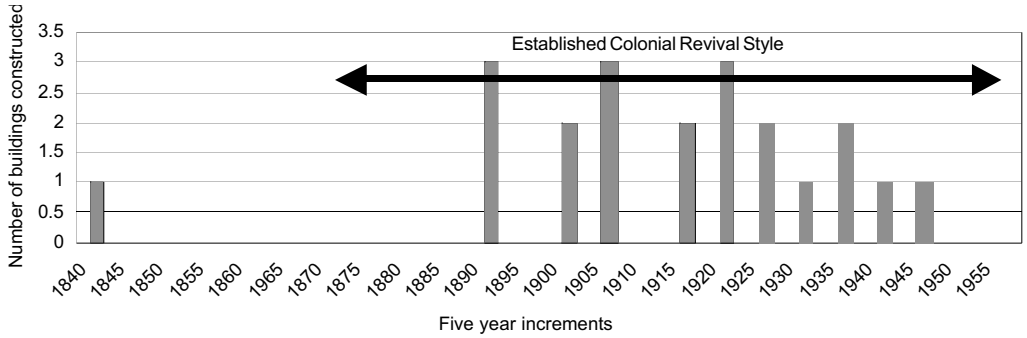


Fig. 1. Architectural trends in Fayetteville, Arkansas.

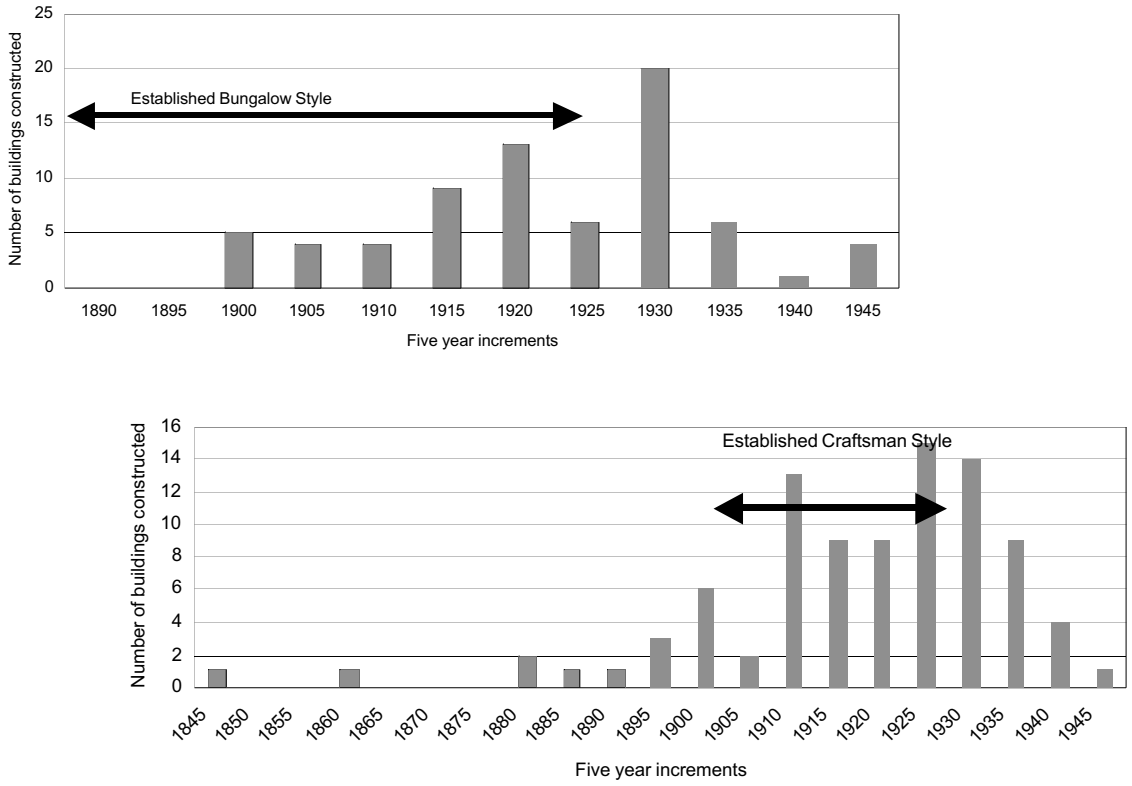


Fig. 1. continued. Architectural trends in Fayetteville, Arkansas.

the larger cultural, economic, and artistic scene; therefore, understanding regional variations in trend adoption is significant to understanding the relationship of Fayetteville, Ark., to the larger national context. It is a further reflection of the citizens of Fayetteville as consumers of popular culture. The project objectives were to 1) document significant architectural styles within designated historical districts and nearby areas, and 2) compare local stylistic trends with national trends to determine fit.

Background

Private individuals, state or local governments, federal offices, or Native American tribes can nominate buildings for addition to the National Register of Historic Places. The initial evaluation includes property categorization (district, site, building), prehistoric or historic context, significance (important event or person, design or construction), determination of exclusion, and integrity (National Register Bulletin, 1998). If the building passes the initial evaluation, a nomination form is used to record the building's features and determine the style and significant history related to the building. The National Register Bulletin (1998) gives explanations of what the Register is and how it works. The Keeper of the National Register is responsible for the final evaluation and listing of the building (National Register Bulletin, 1998).

The earliest recorded reference to Fayetteville is in 1819 (USA Fact File, 2000). Fayetteville was officially established in 1828, and the boundary marking the Indian Territory was moved 40 miles west of the town (Facts and History, 1999). This boundary relocation created the perception that the area was considerably safer, and the city grew in population. Fayetteville achieved town status in 1835, was surveyed into lots sold at public auctions over the next two years, and by 1841 had a population of 425 (USA Fact File, 2000). Fayetteville was incorporated in 1870.

Access to Fayetteville was at first limited to wagon or river transportation. The materials for the first buildings were brought in by wagon. Later, the

Butterfield Stage Line ran through Fayetteville between 1858 and 1861 (Key to the City, 1999); the stagecoach brought settlers, news, and visitors to Fayetteville and provided a major connection between this area and the rest of the nation. The Arkansas Industrial University was established in 1872, and renamed the University of Arkansas in 1874. The establishment of the University attracted faculty, students, and supporting populations to the area, furthering diverse ideas and influences. When the first passenger train arrived in 1882, it marked the end of the geographical isolation previously experienced by the area. In 1925, the rail depot operated by the Frisco Line received a major renovation and expansion as a result of the rapid growth of the area coupled with expansion at the University of Arkansas (Arkansas Historic Preservation Program Records, 1984). This expansion marks a further growth in the area and is significant in the influx of new ideas and information into the town.

MATERIALS AND METHODS

Our sample consisted of 587 structures in three categories. The first category (n=196) included structures listed in 2000 in the National Register for Historic Places; these structures remain accurate representations of architectural style or construction. The second category (n=349) included structures that had been previously surveyed by the Arkansas Historic Preservation Program but had not been listed in the Register due to additions or alterations that impacted the original integrity of the architectural style. The third category (n=42) included buildings considered by the researcher to have historical value as a result of appearance, and located within reasonable proximity to existing structures or districts presently listed with the Arkansas Historic Preservation Program.

A search of the Arkansas Historic Preservation Program records provided the lists of buildings named above, excluding the last category of structures. These records provided construction dates, stylistic classifications, and, in some cases, brief histories of the property or area. All buildings included in the sample were photographed for reference. Additional records searches in the Washington County archives allowed the researcher to establish construction dates of those buildings not surveyed by the Arkansas Historic Preservation Program.

The sample was organized by construction date, primary and secondary style, and physical location within the city. Structures that had no style assigned previously were categorized utilizing standard classification texts (Blumenson, 1983; McAlester and McAlester, 2000; Rifkind, 1980). Houses (n = 217) that had been previously categorized as "traditional" were re-examined and classified according to predominant styles when possible; 110 structures were reassigned to stylistic categories.

Frequency counts were completed for all buildings

Table 1. Comparison of building construction dates in Fayetteville, Ark., to nationally established style dates.

No. of Houses	Style	Date range	Style range *
6	19th C Commercial	1897 - 1930	*
40	20th C Commercial	1900 - 1965	
2	4 Square	1910 – 1925	*
2	Adam	1919-1944	*
8	Art Deco	1925 - 1946	1920 - 1940
77	Bungalow	1897 - 1956	1890 - 1940
1	Cape Cod	1892	*
6	Classical Revival	1901 - 1930	1770 - 1850
5	Collegiate Gothic	1934 - 1939	*
25	Colonial Revival	1840 - 1947	1870 - 1955
6	Cottage	1905 - 1948	*
93	Craftsman	1845 - 1945	1904 - 1930
7	Dutch Colonial	1900 - 1955	1625 - 1840
4	English Revival	1866 - 1940	*
1	Federal 1897	1780 - 1820	
2	French Colonial	1913 - 1930	1700 - 1860
8	Gothic Revival	1871 - 1931	1830 - 1880
33	Greek Revival	1830 - 1950	1820 - 1860
3	Industrial	1908 - 1946	*
10	Italianate	1871 - 1940	1840 - 1885
1	Log	1910	*
1	Mission	1925	*
17	Modern	1950 - 1988	1930 - Present
8	National	1885 - 1940	1850 - 1890
1	Neo-Classical	1907	1895 - 1950
12	Prairie	1850 - 1931	1900 - 1920
66	Queen Anne	1869 - 1948	1880 - 1910
3	Ranch	1959 - 1985	1935 - Present
3	Renaissance Revival	1909 - 1940	1890 - 1935
1	Second Empire	1871	1855 – 1890
1	Shingle	1905	*
107	Traditional	1890 - 1990	*
19	Tudor	1888 - 1946	1890 - 1940
3	Victorian Folk	1885 - 1988	1870 - 1910
1	WPA Rustic	1940	*

Note: Style range was established by averaging the dates established in reference texts. * Style was provided by the Arkansas Historic Preservation Program and style range is not verifiable in reference texts.

in the sample by construction date, style, and number of structures (Table 1). Distribution curves were generated for each style with 10 or more buildings listed in the National Register, which included Greek Revival, Italianate, Colonial Revival, Queen Anne, Bungalow, and Craftsman. A chart was created for each of these styles illustrating the number of structures in the sample constructed in five-year increments. For each of these distribution curves, an overlay illustrating the date range of the style was created (Fig. 1). Stylistic date ranges were established by averaging the date ranges stated in each of the primary reference texts (Blumenson, 1983; McAlester and McAlester, 2000; Rifkind, 1980).

RESULTS AND DISCUSSION

The comparison of Fayetteville's construction date ranges to established stylistic date ranges indicates that Fayetteville, in its early years, could be termed a fashion laggard in the architectural sense. A comparison of the styles established during the early to mid-1800s (Greek Revival and Italianate) indicates that Fayetteville was lagging in the implementation of both styles. The Greek Revival and Italianate styles had a small initiation toward the end of the date range and considerable use as much as 30 to 50 years afterward. Comparatively, plots of the Colonial Revival, Queen Anne, Bungalow and Craftsman styles, initiated in 1875, 1880, 1890, and 1900, respectively, indicate that Fayetteville construction was reflective of the nation at large in the adoption of these four styles. In Fayetteville, the Bungalow style continued well after the official date range; this is reflective of the nation at large. The style was affordable and appealing and responded to a particular lifestyle. Additionally, there was a trend away from clearly defined styles, particularly in residential construction, and the continuation or reintroduction of earlier styles is reflective of this.

Several events in Fayetteville's history indicate that information about architectural style and fashion is tied directly to links outside the region. The path of the Butterfield Stage Line in 1858, the establishment of the University of Arkansas in 1874, and the construction of the railway station in 1881 were events that provided regular delivery of news and the influx of people from other regions of the United States. These events coincide with Fayetteville's shift from laggard to mass market consumer in architectural style. In Fayetteville, as well as many other mid-continental areas of the United States, "fashions" took a long time to be introduced because of the secluded locations.

Several tracks for future investigation can be developed from the data collected. There were 13 houses in which the style postdated the actual construction date, suggesting that significant renovations and additions had been made. Analysis of the renovation dates may illustrate the same delayed curve seen in the styles of other buildings. Yet another study of interest would be to compare Fayetteville to larger cities such as Little Rock or Memphis. Varying transportation methods and access to information may be more clearly defined in those large cities established earlier.

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Sulfur amino acid requirements of broilers from two to five weeks of age

Jill A. Townsend,[*] *H. R. (Trey) Pope,*[§] *and Jason L. Emmert*[¶]

ABSTRACT

Phase-feeding (PF) in broiler chickens has been researched as a way to reduce feed costs without reducing growth performance and yield. Predicted amino acid requirements for PF are generated using linear regression equations derived from best estimates of lysine (Lys), sulfur amino acid (SAA), and threonine (Thr) requirements. During the late starter and early grower periods, predicted requirements for the SAA methionine (Met) and cysteine (Cys) are higher than levels recommended by the National Research Council (NRC), and previous research suggests that SAA may be lowered during the grower period without sacrificing growth performance or yield. The objective of this study was to estimate Met and Cys requirements for broilers from 2 to 5 weeks of age. In Experiment 1, a Met-deficient corn-peanut meal diet was formulated to contain excess Cys, so that supplemental Met was not utilized for Cys synthesis. The basal diet for Experiment 2 met the Met requirement but was deficient in Cys. Graded levels of Met (0, 0.045, 0.09, 0.135, and 0.225%) and Cys (0, 0.035, 0.070, 0.105, 0.140, 0.175%) were added in Experiments 1 and 2, respectively, and diets were fed to five replicates of five broilers per pen. Broken-line analysis was used to estimate SAA requirements. The digestible Met and Cys requirements from 2 to 5 weeks of age were 0.33% and 0.31%, respectively. Requirement estimates were lower than those predicted by PF or recommended by NRC, indicating that lower SAA levels may be utilized in a PF program.

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Meet the Student-Author

Jill A. Townsend

I graduated from Malvern High School as a cum laude graduate, and I am now a senior majoring in Poultry Science. I have received a number of scholarships including the Freshman Academic Scholarship, Randal Tyson Memorial Scholarship, Joseph E. Fleming Scholarship, Governor Homer M. Adkins Scholarship, Woodmen of the World Scholarship, James T. Whitmore Scholarship, John Rust Foundation Scholarship, Cecil Hearn Memorial Scholarship, and the Stephen F. Peters Scholarship. I have also been inducted into the Golden Key National Honor Society, Gamma Beta Phi, and Mortar Board, and I was awarded the Peggy Walker Scholarship from the Arkansas Alumni Association for my dedication to academics, community service and leadership with the Student Alumni Board.

Along with academics, I enjoy working with others in many campus activities, as the Senior Ambassador to the Bumpers College Student Ambassador program, the vice president to the Student Alumni Board, an active member of the Poultry Science Club, a member of an intramural volleyball team, and a member of the championship women's intramural softball team for the spring semester.

During the summer I am working as a Human Resources intern with Wayne Farms in Danville, Arkansas. Upon graduation, I hope to attend graduate school at the University of Arkansas and eventually obtain a position within the poultry industry with a career in research or possibly human resources.

I became interested in research during my summer internship while working with Dr. Emmert and his nutrition trials. Throughout the summer I learned about many aspects of performing a research trial, including the process of developing feed formulations, proper feed mixing procedures, and how to analyze results to an experiment. I chose to participate in this research project because of the invaluable experience I could use towards my studies in graduate school. I have learned a lot about poultry nutrition and the skills needed for performing experiments.

INTRODUCTION

In commercial broiler chicken diets, sulfur amino acids (SAA; methionine and cysteine) are the most deficient of all essential amino acids. Nearly all broiler diets require supplemental methionine (Met) to supply the necessary sources of SAA. As a result of the supplementation, the SAA market continues to be a multimillion dollar industry each year and a serious cost concern for the poultry industry. Information regarding SAA requirements for broilers less than three weeks of age is plentiful, but reliable requirement data beyond three weeks is scarce for the modern commercial broiler. This lack of knowledge about SAA requirements for older birds has led to research with the goal of decreasing feed costs for broiler diets. A better understanding of SAA requirements would provide poultry nutritionists with an opportunity to potentially save money by decreasing the need for over-use of supplemental Met in the broiler diets.

Accurate estimates of SAA requirements for broilers are also needed to fine-tune the phase-feeding (PF) system that is currently under investigation (Loupe and Emmert, 2000; Pope and Emmert, 2001; Warren and Emmert, 2000). Phase-feeding is a concept in which dietary amino acid levels are decreased throughout the starter (0-3 weeks), grower (3-6 weeks) and finisher (6-8 weeks) periods to more closely match a bird's daily

requirement. The steady decrease in dietary amino acid levels potentially provides the poultry industry with a more cost-effective way of growing birds. The PF regimen also addresses environmental concerns by possibly leading to a decrease in nitrogen excretion through elimination of excess amino acids (Loupe and Emmert, 2000). However, predicted SAA requirements for days within the 3 to 6 week age range are higher than those recommended by the National Research Council (NRC, 1994), a broiler diet guideline used by most poultry nutritionists. Previous research (Emmert, unpublished data) has indicated that SAA may be lowered during the grower phase without negatively impacting growth performance or carcass yield. Our objectives were to determine the Met and Cys requirements of commercial broilers from 2 to 5 weeks of age, and to compare these requirements to those predicted by PF linear regression equations.

MATERIALS AND METHODS

All experimental procedures have been reviewed and accepted by the University of Arkansas Institutional Animal Care and Use Committee. Prior to experiment initiation broiler chicks of a commercial strain were obtained from a local hatchery, placed in floor pens with pine wood shavings, and provided with a 23% crude protein commercial starter diet from 1 day to 2 weeks of age. Following a 12-hour period of feed withdrawal, at 2 weeks of age birds were weighed, wing-banded and randomly assigned to experimental diets. During both experimental periods birds from 2 to 5 weeks of age were housed in battery cages with raised wire floors. A 24-hour constant light schedule was maintained and feed and water were freely available. Experimental diets were formulated to meet or exceed NRC (1994) recommendations for all essential nutrients with the exception of Met and Cys. Experimental diets were fed to five replicate pens of five birds each, and at experiment termination birds and feed were weighed to allow calculation of weight gain, feed intake, and feed efficiency.

Because of the metabolic relationship between Met and Cys, two experiments were necessary to allow clear determination of individual amino acid requirements. Methionine is a precursor for Cys, so when Cys is deficient excess Met is converted to Cys. However, Cys cannot be converted to Met, so a Met deficiency cannot be alleviated by excess Cys. In Experiment 1, the Met requirement was evaluated in the presence of excess Cys, so the determined requirement was not confounded by Met conversion to Cys. Graded levels of Met (0, 0.045, 0.09, 0.135, and 0.225%) were added to a Met-deficient corn-peanut meal basal diet (Table 1, 0.22% digestible Met) that contained excess Cys. In Experiment 2, the Cys requirement was evaluated by adding graded levels of Cys (0, 0.035, 0.070, 0.105, 0.140, 0.175%) to a Cys-deficient corn-peanut meal basal diet (Table 1) containing the level of Met determined to be the requirement in Experiment 1.

TABLE 1. Composition of experimental diets.[z]

Ingredient	Percent (%)
Cornstarch	to 100
Corn	58.1
Peanut meal	32.1
Soybean oil	6.00
Dicalcium phosphate	2.00
Limestone	1.00
Vitamins mix[y]	0.20
Mineral mix[y]	0.15
Amino acid[x]	1.47
DL-methionine[w]	0.11
L-cystine[v]	0.25

[z] The basal diet (without supplemental methionine or cysteine) contained 0.22% digestible methionine and 0.25% digestible cysteine.

[y] Han and Baker (1993).

[x] Included a mixture of (g/100 g): L-lysine monohydrochloride, 0.86; L-threonine, 0.30; L-valine, 0.14; L-tryptophan, 0.01; L-isoleucine, 0.17.

[w] DL-methionine was added to the basal diet only in Experiment 2.

[v] L-cystine was added to the basal diet only in Experiment 1.

Data were analyzed as a completely randomized design and all requirements were estimated by the broken line method (Robbins et al., 1979). The number of replications and birds per pen was chosen to support statistical validity. The General Linear Models procedure of SAS was used to conduct analysis of variance (SAS Institute, 1996).

RESULTS AND DISCUSSION

As expected, feed intake and feed efficiency was increased ($P < 0.05$) by the addition of Met or Cys to the basal diet in Experiments 1 and 2, respectively (data not shown). Weight gain was plotted against digestible Met (Fig. 1) and digestible Cys (Fig. 2) con-

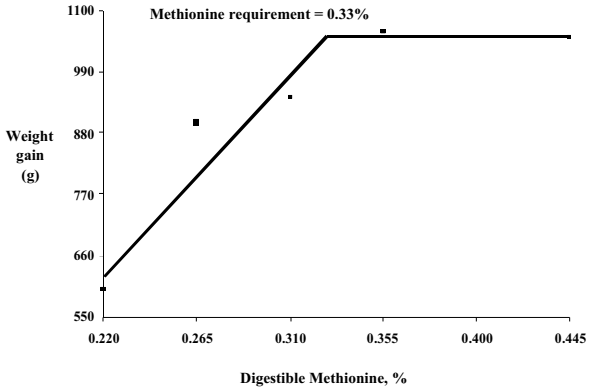

Fig. 1. Digestible methionine requirement of commercial broilers from 2 to 5 weeks of age. Graded levels of methionine were added to a methionine-deficient basal diet and weight gain was plotted versus dietary digestible methionine content (%). Broken-line analysis was used to generate an estimate of the methionine requirement.

centration and broken-line methodology was used to arrive at requirement estimates. In Experiment 1, the level of Met needed to maximize weight gain was 0.33% (Fig. 1). In Experiment 2, the level of Cys needed to maximize weight gain was 0.31% (Fig. 2).

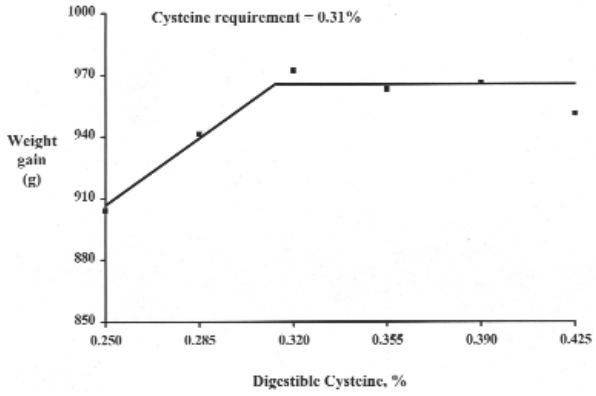

Fig. 2. Digestible cysteine requirement of commercial broilers from 2 to 5 weeks of age. Graded levels of cysteine were added to a cysteine-deficient basal diet and weight gain was plotted versus dietary digestible cysteine content (%). Broken-line analysis was used to generate an estimate of the cysteine requirement.

Determination of SAA requirements can be problematic, due to the complexity of SAA metabolism. Through a series of steps the body can convert Met to Cys; however, the reaction is irreversible, so that no net Met synthesis may occur from Cys. Because Cys can be synthesized from Met it is not considered an essential amino acid, but dietary Cys levels are extremely relevant because if Cys is not present in adequate amounts supplemental Met must be added to allow for synthesis of Cys to cover the dietary shortfall. To further complicate this process, the conversion efficiency of Met to Cys is only 81% efficient (Graber and Baker, 1971). In poultry diets, two conditions usually occur: 1) dietary Cys and Met are both deficient, making Met additions necessary as a source of both Met and Cys (keeping in mind the 81% efficiency), and 2) dietary Cys is adequate or in excess and Met is deficient, making supplemental Met necessary only for the lack of Met. Many previous SAA requirement studies have failed to determine the Cys requirement, which makes interpretation very difficult to determine. Our studies were designed to provide a clear requirement estimate for both Cys and Met.

Our results may be compared with previous SAA requirement estimates, however it must be noted that our trials were conducted from 2 to 5 weeks of age, whereas previous studies have focused on the traditional growth periods of starter (0 to 3 weeks), grower (3 to 6 weeks), and finisher (6 to 8 weeks). Baker and Han (1994) estimated grower period digestible requirements of 0.33% and 0.34% for Met and Cys, respectively. In contrast, NRC (1994) recommendations for the grower period are 0.33 and 0.30%, respectively. Our results clearly showed a lower Cys requirement estimate and a Met requirement similar to that of Baker and Han (1994). Our results showed Met and Cys requirements similar to those listed by the NRC (1994). However, given that amino acid requirements decrease with age (NRC, 1994), one would conclude that our requirement estimates even for Met were slightly lower than Baker and Han (1994) and the NRC (1994), because we had similar requirement estimates at an earlier average age.

In addition to providing accurate requirement estimates for Met and Cys for modern commercial broilers from 2 to 5 weeks of age, our data are pertinent to phase-feeding. Predicted amino acid requirements for PF are generated using linear regression equations derived from best estimates of lysine (Lys), sulfur amino acid (SAA), and threonine (Thr) requirements. However, during the late starter and early grower periods predicted requirements for methionine (Met) and cysteine (Cys) are higher than levels recommended by NRC, largely because predictions are based on the

requirements of Baker and Han (1994). Preliminary data (Loupe and Emmert, 2000) indicated that SAA levels may be lowered in the PF regimen without sacrificing growth performance or yield, and data from these experiments verify that SAA requirements for the period 2 to 5 weeks of age are lower than the requirement predicted by PF linear regression equations.

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Evaluation of chilling requirements for six Arkansas blackberry cultivars utilizing stem cuttings

Dayanee Yazzetti[*] *and John R. Clark*[§]

ABSTRACT

Woody perennial plants including blackberries (*Rubus* subgenus *Rubus*) require certain amounts of chilling or rest hours below 7ºC during the dormant season for successful bud break the following year. Arkansas-developed blackberry cultivars are being grown in various climates worldwide and all cultivars need chilling requirement estimates for accurate recommendations of adaptation. Determining chilling requirement using stem cuttings collected from field-grown plants rather than whole plants is a desirable system. We conducted a study to evaluate both artificial and field chilling of six cultivars. For the artificial-chilling study, 12-node stem cuttings were collected 2 days after the first killing frost. These were then placed in a moist medium in a walk-in cooler at 3ºC. At 100 hour chilling intervals, five cuttings of each cultivar were placed under an intermittent mist system. For the field-chilling study, a biophenometer was placed in the field to measure chill, and ten 12-node stem cuttings of each cultivar were collected at 100-hour intervals of chilling up to 1000 hours below 7ºC and placed under mist. For both studies the mist bench was located in a heated greenhouse (min. temperature of 15ºC), and cuttings were placed according to a completely random design. Budbreak was recorded weekly. Studies were analyzed separately by SAS. Results for Study One, artificial-chilling, were inconclusive due to a lack of clear differentiation among the cultivars and chilling intervals. Study Two, using field-chilling, showed a significant chilling interval x cultivar interaction. 'Arapaho' appeared to have a chilling requirement of 400 to 500 hours, 'Kiowa' 200 hours, 'Shawnee' 400 to 500 hours, and 'Chickasaw' possibly 600 to 700 hours. The cultivars Choctaw and Apache did not provide clear chilling interval differentiation in the study. Our results indicate that the use of stem cuttings receiving field chilling to evaluate chilling requirement of blackberry cultivars has merit and can be a successful method in this research area.

[*] Dayanee Yazzetti graduated in May 2001 with a B.S. degree in horticulture.

[§] John R. Clark, faculty sponsor, is a professor in the Department of Horticulture.

INTRODUCTION

Woody perennial plants such as blackberry require certain amounts of chilling or rest during the dormant season for successful budbreak and normal shoot and flower development to occur during the next season. Rest period is defined as the duration that a plant must be exposed to cold temperatures at or below 7ºC, while chilling requirement is the amount of cold needed to satisfy that rest period and is species and often cultivar specific (Ryugo,1998). Failure to meet this requirement results in reduced and erratic budbreak, poor shoot growth, reduced flowering, and reduced fruit yields the next year.

Blackberry cultivars released from the University of Arkansas breeding program include 'Shawnee' (Moore et al.,1985), 'Choctaw' (Moore and Clark, 1989), 'Navaho' (Moore and Clark, 1989), 'Arapaho' (Moore and Clark, 1993), 'Kiowa' (Moore and Clark, 1996), 'Apache' (Clark and Moore, 1999), and 'Chickasaw' (Clark and Moore, 1999). Arkansas developed blackberry cultivars are being grown not only in Arkansas but worldwide, in locations with different amounts of chilling than where they originated. Chilling requirement estimates are needed for all cultivars to ensure accurate recommendations of adaptation. Limited formal research has been performed on chilling requirement of blackberry cultivars. Drake and Clark (2000), reported chilling requirement of 'Arapaho' was 400 to 500 hours and 'Navaho' was 800 to 900 hours using whole plants in a study with controlled artificial chilling of constant 3ºC.

In the fall of 2000-2001, we conducted two studies to evaluate the use of stem cuttings to estimate chilling of six blackberry cultivars. The first study (Study One) was conducted to determine the feasibility of using artificial chilling to fulfill chilling requirements of stem cuttings. The objective of Study Two was to determine the feasibility of using blackberry stem cuttings receiving natural chilling to identify chilling requirement.

MATERIALS AND METHODS

Study One

Fifty 12-node, lateral-branch stem cuttings of 'Apache', 'Arapaho', 'Chickasaw', 'Choctaw', 'Kiowa', and 'Shawnee' were collected from a mature planting located at the University of Arkansas Agricultural Research and Extension Center, Fayetteville, 2 days after the first killing frost, 12 Oct. 2000. The cuttings were then placed in a moist sawdust medium in a walk-in cooler at 3ºC. At 100 hour chilling intervals, five cuttings of each cultivar were removed from the cooler and placed under an intermittent mist system in a completely random design. The mist bench was located in a heated greenhouse with a daily minimum temperature of 15ºC and a daily maximum temperature of 25ºC.

Study Two

In order to measure natural field chilling, a biophenometer was placed in the planting to record the number of hours below 7ºC. Ten stem cuttings from lateral branches of mature canes of each of the cultivars mentioned above were collected from the field at 100-hour intervals of chilling up to 1000 hours. However, due to a severe ice storm in December, the 900 hour chilling interval cuttings were not taken due to the inability to collect the cuttings. Also, 'Arapaho' cuttings were only collected for 100 to 600 hours of chilling due to the shortage of lateral branches in the planting for this cultivar. Following collecting, the field cuttings were placed in the same greenhouse under an intermittent mist system in a completely random design. For both studies, incandescent lighting was provided to lengthen the daylength to 16 hours in the greenhouse.

Data collection for both studies consisted of a budbreak count of each cutting of each cultivar weekly for 10 weeks. A bud was considered broken when the first leaf became visible as it unfolded from the bud. Budbreak data after 10 weeks for each study were analyzed separately by SAS (SAS, 1989) and standard errors of the means calculated.

RESULTS AND DISCUSSION

Study One

The data analysis for Study One indicated a significant chilling interval x cultivar interaction, indicating that the cultivars did not have the same budbreak for all chilling intervals. For 100 to 600 hours, all cultivars except 'Kiowa' had 15% budbreak or less, indicating chilling differentials did not appear to be delineated using the artificial chilling method (data not shown). Substantial budbreak was experienced at several higher chilling (above 600 hours) levels for 'Choctaw', 'Apache', and 'Shawnee'. However, 'Arapaho' had very low budbreak for all the intervals except 900 to 1000 hours, and this result contradicts that of Drake and Clark (2000), who estimated 'Arapaho' chilling of 400

to 500 hours. 'Kiowa' behaved differently from all the other cultivars, showing no lower than 20% budbreak across all intervals and increasing up to 70% for the 1000 hour chilling interval. The lack of comparable findings for 'Arapaho' as reported before, and the lack of clear differentiation among the chilling intervals of the cultivars, indicated that this method was likely not a reliable method for chilling requirement estimates.

Study Two

The chilling interval x cultivar interaction was significant for this study, indicating that budbreak differed among the cultivars for the various chilling intervals. 'Arapaho' was the only cultivar with a known chilling requirement used in the study, and it had a substantial increase in budbreak between 400 and 500 hours, consistent with the findings of Drake and Clark (2000) (Fig. 1). This finding was very important as it shows that this method of chilling determination appeared to

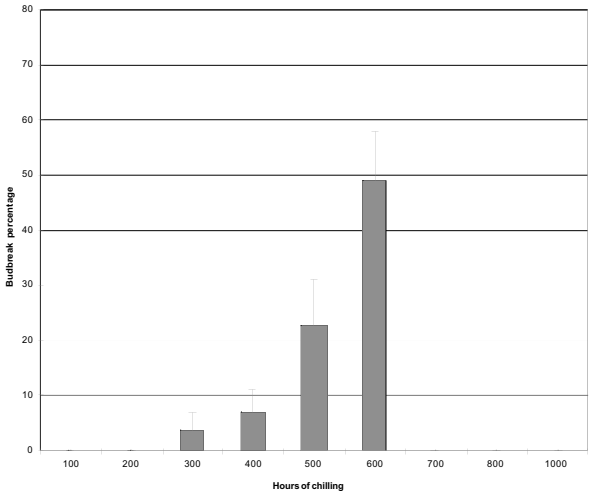

Fig. 1. Budbreak of 'Arapaho' blackberry after 10 weeks of forcing in a heated greenhouse following 100 through 1000 hours of chilling, below 7°C.

be successful for this cultivar. 'Kiowa' had substantial budbreak at 200 hours, and at most other chilling intervals (Fig. 2). There was a reduction in budbreak at 300 hours for 'Kiowa', due to the death of several cuttings collected for this chilling interval contributing to the low budbreak value. There was a substantial reduction for 'Kiowa' at the 800 and 1000 hours, likely due to winter injury sustained from extreme low temperature (-16.7ºC) during this chilling interval. Based on these finding it appears that 'Kiowa' has the

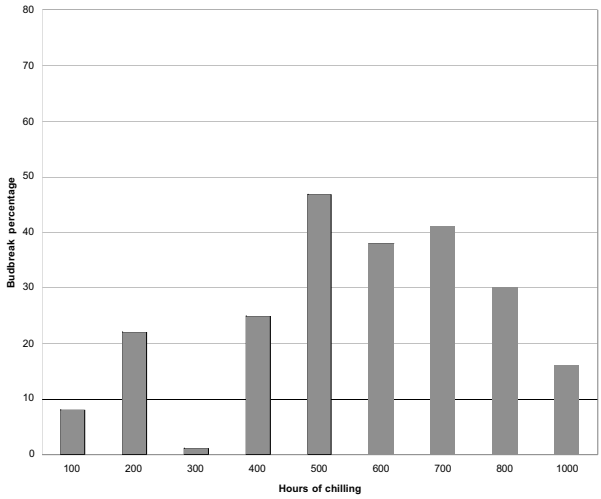

Fig. 2. Budbreak of 'Kiowa' blackberry after 10 weeks of forcing in a heated greenhouse following 100 through 1000 hours of chilling, below 7°C.

lowest chilling requirement of the Arkansas cultivars, and this may be a low as 200 hours.

Field observations of 'Choctaw' in more subtropical climates of the world have shown it to have a lower chilling requirement than other Arkansas cultivars released prior to 1989 (J.N. Moore, personal communication). In Study Two, 'Choctaw' showed no budbreak until 400 hours, with higher budbreak at other chilling intervals (data not shown). Budbreak never exceeded 32% for 'Choctaw' at any interval, however, which was lower than most other cultivars. We conclude that for 'Choctaw' data were inconclusive in substantiating the low chilling observations that have been reported previously.

'Shawnee' has been the most widely grown Arkansas blackberry cultivar, with widespread planting of this cultivar in the southern U.S. Occurrences of evidence of lack of chill have not been reported (J.N. Moore, personal communication). In our study, 'Shawnee' appeared to have a chilling requirement of 400 to 500 hours due to the greatly increased budbreak between these two intervals (Fig. 3). Since most of southern states receive this amount or more chilling, one would expect a cultivar to not experience chilling requirement shortfalls at this chilling level. The chilling requirement seen in our data support this observation. The two newest Arkansas cultivars, 'Apache' and 'Chickasaw', have no chilling observations available. 'Chickasaw' had substantial budbreak at 700 hours of 50%, a major increase in budbreak compared to lower chilling intervals (data not shown). This suggests

Meet the Student-Author

I am a 1997 graduate of Mena High School and a 2001 graduate of the University of Arkansas with a bachelor's degree in horticulture. I have been actively involved with the Horticulture Club since my arrival on campus four years ago. I have been the recipient of numerous scholarships, among them being the Gerber Endowment Scholarship and the Arkansas State Horticulture Society Scholarship. I have also been awarded the 1st Place Undergraduate Research Presentation by the Southern Region American Society for Horticultural Science, and the Gamma Sigma Delta's 2nd Place Award in their annual Undergraduate Research Presentation competition.

I will begin attending Graduate School in the Fall of 2001 here at the University of Arkansas.

While working on my internship at the UofA's Fruit Substation in Clarksville, Dr. Clark suggested the idea for my research. Since little research had been done on the chilling requirements of Arkansas-developed blackberries, I was intrigued by the opportunity of being involved in potentially groundbreaking research.

Dayanee Yazzetti

'Chickasaw' has a chilling requirement possibly between 600 to 700 hours. Budbreak did not remain as high for 'Chickasaw' at 800 and 1000 chilling intervals, which again might be due to winter injury to some buds. Finally, 'Apache' had low budbreak at all chilling intervals, with the highest level at 800 hours of 20% (data not shown). It was anticipated that 'Apache' would have chilling near to that of 'Navaho' (800 to 900 hours as found by Drake and Clark, 2000), as 'Navaho' is one of its parents. Due to the low budbreak at all intervals, we feel our results are inconclusive in estimating chill requirement for 'Apache'.

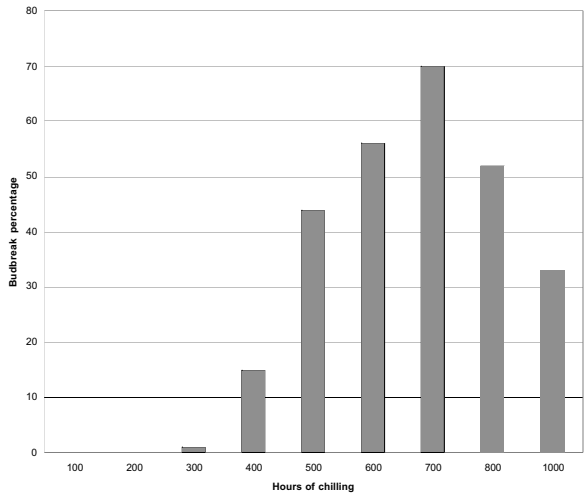

Fig. 3. Budbreak of 'Shawnee' blackberry after 10 weeks of forcing in a heated greenhouse following 100 through 1000 hours of chilling, below 7°C.

The major premise of our studies was to determine if the use of stem cuttings would be successful in differentiating chilling requirements of blackberries. Stem cuttings are much easier to use for chilling requirement determinations as they can be collected from field-grown plants and forced to budbreak after collection. Conversely, using whole plants for this type of research requires that potted plants be grown for a season prior to exposure to chilling, and then that the whole plants be used for budbreak measurements after chilling treatment intervals are provided. This is a much more laborious and expensive process. Also, before or near the release of a new cultivar there is often a very limited

number of plants available, and having whole plants to use in a chilling determination study is usually not possible. However, using stem cuttings, which are much more plentiful in research plots, would be much more practical. Therefore, the evaluation of stem cuttings was deemed necessary as a method to investigate.

The use of artificial chilling on blackberry stem cuttings (Study One) was deemed unsuccessful in our study due to the lack of differentiation among most cultivars, and the low budbreak at all of the lower chilling intervals except for 'Kiowa'. This could be due to several reasons. It is possible that the cuttings were collected prior to the onset of dormancy of the plants. When dormancy actually begins is always a question, and we are not aware of an absolute way to know this. Our collection was based on the occurrence of the first killing frost on 12 Oct., which we hoped would be the beginning of dormancy or rest period. However, if the plants were not physiologically in or near dormancy at this time, this could affect subsequent ability of the plant to show response to chilling to satisfy the chilling or rest period requirement, and this could have contributed to our inconclusive results. Also, the plant material may require attachment to an entire plant to allow the measurement of chilling to fulfill the rest period, and this may not have been possible when the stem cuttings were removed from the plant. Whether the reasons are those discussed here, or the results were due to other causes, we feel that artificial chilling of stem cuttings was not a reliable method to measure chilling requirement of blackberries.

Conversely, the field-chilling study (Study Two) provided results that we feel allowed the differentiation of chilling requirement of most cultivars. Previous research by Drake and Clark (2000) showed a difference among two Arkansas cultivars in chilling requirement, and field observations in areas of low chill had also indicated cultivar chilling requirement differences. Our first noteworthy finding, that of a similar estimate of chilling response of 'Arapaho' stem cuttings exposed to field chilling compared to that found by Drake and Clark (2000) using whole plants of 400 to 500 hours, provided confidence in the stem cutting method we used.

A very apparent additional finding in Study Two was the unusual budbreak at low chilling level for 'Kiowa'. This cultivar was released in 1996, and has not been planted as widely as yet as cultivars such as 'Shawnee', 'Choctaw' or 'Arapaho'. Therefore, reports from growers and researchers have not surfaced as to its chilling response, possibly because of the rather short period of time 'Kiowa' has been planted on a widespread basis. It was observed in the testing of 'Kiowa' prior to its release that it had earlier spring budbreak compared to 'Shawnee' and 'Choctaw' (Moore and Clark, 1996), and this might reflect either a lower chilling requirement or a lower hear requirement for bud development. Our data support the idea that this could be due to a lower chilling requirement, as our study did not measure differential heat requirement conditions. Additionally, a reason that no chilling concerns have been observed by early evaluators of 'Kiowa' may be due to the fact that it has had good budbreak in all areas grown, both low and high chill locations, due to its low chilling requirement. We conclude that 'Kiowa' likely has the lowest chilling requirement of all cultivars tested in our study.

We expected a low chilling requirement response for 'Choctaw' based on field observations of its reliable budbreak in locations of low chill. Our data were disappointing as we observed rather low budbreak at all chilling intervals, and therefore the differentiation of these was not reliable. Reasons for this were not clear, but could include the possibility of cold injury to buds during the study, or could be related to heat requirement to begin growth. 'Choctaw' has been observed to be the least hardy (most susceptible to winter injury) of the Arkansas cultivars (J.N. Moore, personal communication), and it is possible some bud injury occurred early in the fall. However, bud injury was not evaluated at collection thus this suggestion cannot be confirmed. The heat requirement for growth to begin has not been measured for any Arkansas blackberry cultivars, thus it is not possible to speculate if this was involved in our study, as the environment in which the cuttings were forced was thought to be warm enough to contribute to budbreak for all cultivars.

'Shawnee' response was very much as expected, as a chilling requirement of 400 to 600 hours was suspected for this cultivar based on field performance. Our finding of a requirement of 400 to 500 hours fell within this expected range, and the budbreak levels were among the highest of all cultivars after these chilling treatments. This provided further confidence in our method.

Finally, the results for 'Chickasaw' indicate that it might have a higher chilling requirement than 'Shawnee' by 200 hours. Further research and observa-

tion should be done on this cultivar to substantiate the chilling requirement of this new cultivar. 'Apache', with budbreak below 20% at all intervals, needs further investigation to determine chilling requirement. Why differentiation of chilling was not achieved in our study with this cultivar is not understood, as we were not aware of any limitations this cultivar had, such as winter injury of buds prior to collection, heat requirements, or other causes.

In conclusion, our results indicate that for the majority of the cultivars evaluated in our study, the use of stem cuttings receiving field chilling was a successful method of chilling requirement determination. We suggest that this investigation be repeated to verify this, and that bud viability of cultivars be determined prior to forcing to verify that winter injury does not contribute to reduced budbreak. Additionally, with other fruit crops, including peaches (*Prunus persica* Batsch.), it has been reported that different temperatures contribute to efficiency of chilling requirement fulfillment (Richardson et al., 1974). With peaches, temperatures between 7 and 0ºC provided the most efficient chilling, while temperatures below 0ºC contributed to little chill requirement fulfillment. The efficiency of chilling of various temperature ranges should also be investigated on blackberry to determine if a similar response is involved.

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