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Cover: Features the research of Bumpers student Alessandro Rocchi, a Poultry Science major, who conducted research on the effects of cyclic heat stress on immune system function in broiler chickens. (U of A System Division of Agriculture photo by Fred Miller). Alessandro and Dr. Erf, his faculty mentor, discuss their research relationship on pages 5–6.
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Call For Papers

Instructions for Authors
In my position as Senior Associate Vice President for Agriculture – Research and Director of the Agricultural Experiment Station, I have a great appreciation for the outstanding research being conducted by our faculty members and scientists.

In my position as interim Dean of U of A’s Dale Bumpers College of Agricultural, Food and Life Sciences, I also have a great appreciation for the research being conducted by our students as they take advantage of opportunities to work under the mentorship of our faculty and scientists.

Efforts in both of these areas of teaching and research, along with service and outreach, help us meet our land-grant mission.

Our students have a wide range of career options to pursue, and with that many directions they can choose in terms of research.

Research allows students to ask questions, gather data, and answer questions and solve problems impacting everyone.

This year’s issue of Discovery, our undergraduate research journal which allows students to publish their work in a citable publication, includes eight projects across seven different majors and disciplines.

You will see projects and findings from the areas of human nutrition and dietetics, birth through kindergarten, and human development and family sciences in our School of Human Environmental Sciences; poultry science (two), animal science, horticulture, and environmental, soil and water science.

It is inspiring and gratifying to see the results of hard work completed as our students have investigated questions and problems, and reached conclusions with potential answers and solutions.

We are here to serve the people of Arkansas, the entire country and the world, and you will see our students are in a position to do so as their findings are published and they prepare to embark on their professional careers.

This issue of Discovery highlights the efforts of just a few of them. Our faculty work with them to produce what you see because they care about students and their development, as well as the research and its impact.

We encourage undergraduate research by awarding undergraduate research grants. Our students compete for research and travel grants awarded by the University of Arkansas Honors College and the Arkansas Department of Higher Education SURF grants program.

Projects may be designed to meet requirements for an honors project in the Bumpers College Honors Program. One of our goals is to prepare students to be responsible leaders with strong communication skills and problem-solving abilities. You have results of studies highlighting and exemplifying those qualities in our student researchers and future leaders.

Congratulations to the student authors on completing these projects. Thank you to the faculty mentors and editors who worked with them to make this collection possible. As a college, we are pleased and proud to present this collection as a service to them and our readers.

Jean-François Meullenet, Interim Dean
Dale Bumpers College of Agricultural, Food and Life Sciences
Letter from the Faculty Editor

This is the 5th year that I have had the honor of serving as editor of Discovery. I continue to be impressed with the variety and timeliness of these projects that undergraduate students are conducting in the Dale Bumpers College of Agriculture, Food and Life Sciences (DBCAFLS), often in conjunction with the University of Arkansas System Division of Agriculture’s Arkansas Agricultural Experiment Station.

Faculty from throughout the DBCAFLS take on an additional role as mentors to students conducting research and completing these special projects. Their efforts are to be commended and I hope they realize the often life-altering impact these experiences have on the students. I encourage you to read the letters from Dr. Gisela Erf and Alessandro Rocchi with their insights as a mentor and mentee, respectively. While the input of the mentor is vital, this works in conjunction with each of these student’s own desire to persevere and accomplish these projects, as stated in the journal’s instructions the “expectations are that the student(s) has gone above and beyond the requirements of literature reviews and is generating a new contribution to the field/discipline.” These eight papers represent significant effort by both mentor and mentee.

I must also acknowledge the work of Gail Halleck, the journal’s technical editor. In preparing this publication, she works extensively with the students from questions regarding manuscript submissions, through multiple revisions, and finally the production of this final product. For many students this is their first experience with publishing a manuscript, Gail’s patience, organizational skills, and intelligence are greatly appreciated. Finally, thank you to the Editorial Board who represent most disciplines in the college, conduct final reviews, and establish guidelines for future publications.

This year’s Discovery is an indication of the amazing things occurring in the DBCAFLS. The faculty’s willingness to provide these experiences and challenge the students is admirable. These students’ work ethic and maturity in submitting their work for critical review and then thoughtfully responding and editing that work is evidence of their bright future. I am proud of their results, and I hope you enjoy and learn from this year’s publication.

Beth Kegley, Faculty Editor, Discovery Journal and Professor, Department of Animal Science
Providing undergraduates with experiential learning opportunities is the most enjoyable and effective teaching experience of my now more than 35 years of teaching and research. Whether teaching structured laboratory courses, training undergraduate research assistants, or mentoring undergraduate research and Honors projects, these interactions provide students with an important context to the variety of subjects covered in their academic programs and insight into real-life application of their learning. As a research mentor, I greatly benefit from these interactions, from staying connected with the undergraduate programs, their challenges, and opportunities, to helping shape novel ideas and pursuing new research directions. Additionally, most of the undergraduate research projects I mentored generated preliminary or additional data for publications and research grants.

This was the case with Alessandro’s project. He was already conducting research on the effects of cyclic heat stress on broiler production with Dr. Billy Hargis’ research team when he approached me about his idea to investigate the effects of cyclic heat stress on immune system function in broiler chickens. Specifically, he wanted to know how the natural defenses, like the acute inflammatory responses, are affected by heat stress conditions. Once agreed to go forward with this idea for his Honors project, Alessandro prepared a well-researched project proposal, successfully applied for research funding from the Honors and Bumpers Colleges, set-up the animal experiments, including daily care and facility maintenance, prepared all the materials, and participated fully and enthusiastically in all aspects of treatment application, sample collection, and processing. Some samples had to be analyzed the day of collection, requiring long days and teamwork with all members of my research group, while he independently carried out analyses of preserved samples over many months post collection. Alessandro did an outstanding job, carrying out all aspects of his ambitious research project and thesis efficiently with great care and precision.

I am so very proud of his accomplishments and thankful for the opportunity to have worked with this talented and resourceful student as mentor, advisor, and teacher. Our student-mentor teamwork has led to a new research direction and funding for my program, and, most importantly, contributed to Alessandro’s pursuit of graduate studies at Clemson University and continued commitment to a career as a scientist and researcher.

Gisela Erf, Professor, Tyson Professor in Avian Immunology, University of Arkansas System Division of Agriculture’s Department of Poultry Science
Being a part of the honors program was one of the most fruitful opportunities in my four years at the University of Arkansas. Completing my undergraduate research at Dale Bumpers College allowed me to feel accomplished and unquestionably proud of what I was able to achieve during my time there.

As a sophomore, I chose Dr. Gisela Erf in the department of Poultry Science as my mentor and given the opportunity, I would do it all again. Dr. Erf served as a superb mentor, kick-starting me into a newfound love for research. She went above and beyond to truly make my experience unforgettable and enjoyable throughout my entire research project. Working with Dr. Erf gave me a small window into what it feels like to be a graduate student, giving me the liberty to conduct my own research, guiding me along the way, and providing me with a love for basic sciences. With this, I was able to learn what a healthy student-advisor relationship should look like and how to really connect with your professors.

Other than with my research, I was able to learn how to navigate the world of academia. Dr. Erf helped me to develop soft skills in communicating and collaborating with others. With this, I was also able to learn a plethora of skills both in the lab and my academic writing for my future endeavors. Pursuing my undergraduate research also played a large influence on my career path as I now depart from the University of Arkansas to pursue a Master’s at Clemson University. Along my academic journey I will always carry that which I have learned under Dr. Erf’s mentorship. My experience as a part of the Dale Bumpers College honors program has been genuinely unforgettable.

Alessandro Rocchi, May 2023 Honors graduate in Poultry Science

IN THE LAB: Alessandro in the University of Arkansas System Division of Agriculture's John Tyson Center For Excellence in Poultry Science Lab using a light microscope and counter to perform differential leukocyte counts on Wright-stained blood smears from broilers to measure the percentages of each white blood cell type. Photo by Aaron Forga.
Discovery on ScholarWorks@UARK

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https://scholarworks.uark.edu/discoverymag/

Bumpers College undergraduate student research reaches a worldwide audience via this powerful database, with its extensive search engine and analytics, and ease in downloading individual articles. Here’s a peek at readership distribution across the globe and most popular Discovery articles by download in recent months.

Table 1. Download history (top 10) for all Discovery issues from the period of 01 July 2022 to 30 June 2023.

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I graduated in May 2022, majoring in Human Nutrition and Dietetics. I grew up in Farmington, Arkansas, graduating from Farmington High School with interests in many different career paths. After beginning my college career as a nursing major and taking multiple nutrition classes, I became very interested in how food and nutrition can make lasting impacts on individuals’ lives and decided to pursue a career in the field of nutrition. Throughout my college career, I have held multiple jobs and positions, including getting the opportunity to work as a lab assistant at the Center for Human Nutrition, a Career Peer Mentor, and involvement in multiple student organizations. These experiences have helped shape me into who I am today. I would like to thank my honors mentor, Dr. Aubree Hawley, for her guidance and support throughout the past two years. Additionally, I would like to thank Dr. Sabrina Trudo and Dr. Jamie Baum for serving on my honors committee, and the Arkansas Department of Higher Education and Bumpers College for their funding for this project.

Meet the Student-Author

Sydney Boudrey

Research at a Glance

- Due to increasing obesity rates, there is a need for dietary interventions to lessen the health impacts and improve overall well-being.
- We tested two specific dietary interventions congruently being time-restricted feeding (TRF) and protein supplementation.
- Overall sleep and mood were unchanged throughout the study in both intervention groups.
Effects of Time-Restricted Feeding and Whey Protein Isolate Supplementation on Dietary Intake, Mood, and Sleep in a 12-Week Randomized Controlled Trial

Sydney E. Boudrey* and Aubree L. Hawley†

Abstract

Obesity affects adults in the United States, leading to chronic diseases and reduced well-being. Time-Restricted Feeding (TRF) is a type of dietary intervention lacking current data regarding the effectiveness on facets of well-being. This study’s objective was to determine the effect of time-restricted feeding supplemented with whey protein isolate on food intake, sleep, and mood in overweight or obese adults. Nineteen participants were randomly assigned to the control or experimental group: 1) control, TRF, and 2) experimental, TRF with whey protein supplementation. Participants followed the assigned dietary intervention for 12 weeks. Every 4 weeks (baseline, week 4, week 8, and week 12), anthropometrics, including height and weight, were measured along with the Pittsburg Sleep Quality Index (PSQI) questionnaire, Profile of Mood States (POMS) questionnaire, and dietary record results. Additionally, ActiGraphy measured objective sleep quality at week 1 and week 12. There were no differences between the control and protein groups regarding sleep and mood parameters. The PSQI results indicated no difference in sleep between groups. The POMS subscores for tension-anxiety, when controlled for baseline, were different, with a decrease in the protein group compared to the control at week 12 ($P < 0.01$). Total food consumption was similar between groups. The results suggested whey protein isolate supplementation with TRF may improve outcomes of mood with no effect on sleep. Therefore, a need for further research to investigate the benefits of TRF and protein supplementation on sleep and mood is necessary.

* Sydney Boudrey is a May 2023 honors program graduate with a major in Human Nutrition and Dietetics.
† Aubree Hawley, the faculty mentor, is an Instructor in the School of Human Environmental Sciences.
**Introduction**

Currently, more than two-thirds of adults in the United States are overweight or obese, and all states and territories have an adult obesity rate of over 20% (CDC, 2022). Not only does obesity increase the risk of chronic disease, but it also has major impacts on well-being, including sleep and mood (Fatima et al., 2016; Luppino et al., 2010). According to the Centers for Disease Control (2018), well-being is an encompassing concept to describe one’s overall life satisfaction in many aspects, including sleep and mood.

Evidence suggests that the associations between mood and sleep are bidirectional, and changes in diet may ameliorate poor sleep and mood via shifts in body composition (Watson et al., 2015; Vashadze Sh, 2007; Milaneschi et al., 2019; Luppino et al., 2010; Kahn et al., 2013). While there are many methods that are used to encourage weight loss, most are associated with increased hunger and reduced fullness (Nickols-Richardson et al., 2005).

Research suggests that high-protein diets may promote weight loss by influencing energy balance and improved body composition in obese adults (Simonson et al., 2020). For instance, increased protein consumption can be linked to higher levels of satiety and energy expenditure (Smeets et al., 2008). Moreover, protein quantity and quality are suggested to influence the effectiveness of dietary protein as a treatment strategy for obesity. While most Americans receive the proper amount of protein based on the Recommended Dietary Allowance, the quality of the protein is also an important factor to consider.

Overall, animal proteins such as beef and milk protein stimulate muscle protein synthesis more effectively than plant-based proteins such as soy (Volpi et al., 2013). One example of a high-quality protein widely used in weight loss interventions is dairy, specifically whey protein isolate (WPI) (Hoffman and Falvo, 2004). Time Restricted Feeding (TRF) is another dietary pattern that may promote weight loss through caloric restriction. To our knowledge, long-term manipulation of macronutrients while following a TRF regimen has not been extensively studied. Therefore, the objective of this study is to determine the effect of time restricted feeding supplemented with whey protein isolate on food intake, sleep, and mood, in overweight or obese adults.

**Materials and Methods**

Subjects were recruited through the university digital newspaper, advertisements on the Center for Human Nutrition website, the Food Science Department website, social media, and by word of mouth. Candidates were phone interviewed to meet the following requirements: must not have taken supplements that may interfere with metabolism, no food allergies, non-smoking, consumed alcohol less than four times per week, non-breastfeeding, not used illicit drugs, or have dieted in the past three months. A total of 19 participants completed the study, 10 were in the intervention group, and 9 were in the control group. All participants were overweight or obese (body mass index, BMI ≥ 25 kg/m²).

Participants signed a consent form following a complete explanation. The protocol was submitted and approved by the University of Arkansas Institutional Review Board before subjects were recruited. The 12-week study was conducted as a randomized control trial with one control group and one dietary intervention group. Participants were randomly assigned to the control group or experimental group: 1) control, TRF (n = 10), and 2) TRF with powdered whey protein supplementation (20 grams/day; n = 9).

Protein supplements were allocated in powder form to individual sachets and were consumed at the breaking of the fasting period each day, and both groups followed a TRF dietary intervention (8-hour eating window with a 16-hour fast) and ate ad libitum. Participants followed the assigned dietary intervention for 12 weeks. Subjects came to the University of Arkansas System Division of Agriculture’s Center for Human Nutrition for sample collection and measurements to be taken. Anthropometrics, including height and weight, were measured. Participants were asked to complete two questionnaires, the Pittsburg Sleep Quality Index (PSQI) and Profile of Mood States (POMS) questionnaire. Results were measured every 4 weeks (baseline, week 4, week 8, and week 12) at the Center for Human Nutrition. Objective sleep quality was measured via an accelerometer at baseline and week 12.

Participants were provided with a booklet that corresponded with their dietary intervention. The booklet provided a guide for TRF and example schedules that the participants could follow. Details for the Actigraph sleep monitor, sleep diaries, and instructions for filling out food records were also included. Booklets given to participants in TRF and WPI supplementation groups included a section with easy and quick recipes for protein supplementation consumption.

Objective sleep quality and duration were assessed via an Actigraph triaxial wrist accelerometer, a validated method of sleep assessment. Participants wore the Actigraphs for one week before the start of the study (baseline) and one week prior to their final visit (week 12). A 7-day average was calculated for each sleep outcome (sleep efficiency, sleep latency, wake after sleep onset, number of awakenings, and length of awakenings). While wearing the Actigraph, participants kept a sleep diary to confirm their sleep schedule and awakenings. Subjective sleep quality was self-assessed using the Pittsburg Sleep Quality Index (PSQI) questionnaire. This questionnaire is the most widely used and accepted for subjective sleep quality (Fabbri et al., 2021). A compiled global score of the seven scored sleep
Fig. 1. POMS Scores. (A) The mean tension-anxiety score of control and treatment groups. Significance was found for treatment ($P = 0.0124$). (B) The mean depression-dejection score of control and treatment groups. (C) The mean of anger-hostility of control and treatment groups. (D) The mean fatigue-inertia of control and treatment groups. (E) The mean confusion-bewilderment of control and treatment groups. (F) The mean total mood disturbance (TMD) of control and treatment groups. ns = not significant. AU = arbitrary units.
**Fig. 2.** PSQI Scores. (A) The mean PSQI Global Sleeping score (GSS) of control and treatment groups. (B) The mean sleep latency score of control and treatment groups. (C) The mean sleep duration of control and treatment groups. (D) The mean habitual sleep efficiency control and treatment groups. (E) The mean sleep disturbances of control and treatment groups. (F) The mean total daytime dysfunction of control and treatment groups. *ns* = not significant. AU = arbitrary units.
components (sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleep medication, and daytime dysfunction) distinguished good sleepers (≤ 5) from poor sleepers.

We administered the Profile of Mood States (POMS). This survey has been used since 1971, and exhibits construct and predictive validity of the 6 POMS subscales (McNair et al., 2003). The test contained a one-word adjective of mood to measure and identify six affective states. The six identifiable mood/affective states were tension-anxiety, depression-dejection, anger-hostility, vigor-activity, fatigue-inertia, and confusion-bewilderment. The Total Mood Disturbance (TMD) score is calculated by summing the scores across all six factors (weighting vigor negatively). Higher subscores for all affect states, but the vigor domain represents poorer mood. The TMD score is the most clinically relevant and ranges from -32 (best possible score) to 200 (worst possible score). Mood was quantified with a 5-point Likert scale by participants.

Each participant completed a 3-day (2 weekdays and 1 weekend day) food intake record every 4 weeks of the intervention. Food records were reviewed and input into the Nutrition Data System for Research (NDSR) software. Total energy intake, macronutrients, total dietary fiber, total saturated fatty acids, total trans-fatty acids, omega-3 fatty acids, and essential amino acids, including tryptophan amounts, were analyzed immediately following 3-day food record collection via the NDSR software providing a report and analysis of nutrient consumption.

Student t-tests were used to determine the difference between the control and intervention groups in participant characteristics, including age, weight, PSQI GSS, POMS TMD, and sleep efficiency at baseline. Repeated-measures analysis of variance (ANOVA) was used to determine the differences in height, weight, food intake, Global PSQI scores, POMS scores, and wrist actigraphy over the course of the 12-week period. Two-way ANOVA was used to determine the difference between the beginning and end of the intervention. All tests were two-sided with P-values ≤ 0.05, indicating significance. Analysis of data was conducted by using the statistical software GraphPad Prism version 9.0.

Results and Discussion

A total of 19 participants were included in the final data analysis (n = 14 females) (n = 5 males). The control group had a lower BMI (P = 0.04) and weight (P = 0.03) when compared to the protein group at baseline; however, there were no differences in height, global sleeping score, total mood disturbance, or sleep latency between groups at baseline.

Results from POMS scores are presented in Fig. 1. There was a difference in treatment for tension-anxiety scores, with the protein group having lowering scores over the duration of the study. Additionally, anger-hostility scores decreased over time in both groups after controlling for baseline. Furthermore, total mood disturbance (TMD) scores exhibited a decrease in both groups at week 12 compared to baseline, with the control group having lower TMD at week 12 (P = 0.03). There were no other differences in results including depression-dejection, anger-hostility, fatigue-inertia, confusion-bewilderment, and total mood disturbance. This data provides insight into potential mood outcomes of individual's TRF in a larger study.

Figure 2 depicts PSQI global sleeping scores and sub scores. Significance was found in treatment of sleep latency (P = 0.02), as the protein group’s scores started below and stayed below the control. When sleep latency was controlled for baseline, it increased in the control group compared to the protein group at week 4 and week 8 with no differences at week 12 (P < 0.01). No other statistical significance was found between global sleeping score, sleep duration, habitual sleep efficiency, sleep disturbances, and total daytime dysfunction. Some studies indicate that increased protein consumption can lead to better sleep quality. For example, Sutanto et al. (2022) found an association between dietary tryptophan levels and sleep quality. Conversely, in a study conducted by Kim et al., 2020, there were no differences in structure of sleep in a 4-week TRF intervention in metabolically healthy adults among a Korean version of the PSQI. Similarly, in our study, the results from the PSQI did not yield differences.

Sleep actigraphy results were compared at week 1 and week 12. There was a difference in the protein group that had a higher awakening length compared to the control group (P < 0.01). There was no time effect nor treatment effect on total sleep time or total minutes in bed. However, there was a group × time interaction (P < 0.05). No other results were different, including sleep latency, sleep efficiency, and wake after sleep onset.

The amount of protein consumed by the protein group was greater than the control group (P < 0.01), as well as an increasing trend for tryptophan (0.09). There was also a group effect for total energy consumed; however, when total energy consumption and protein intake values were controlled for baseline, there were no differences. All other dietary intake remained similar throughout the study. Previous studies investigating a high protein diet had consumption of between 25% to 35% of protein; therefore, the protein supplement consumption may not have been adequate to see results (Smeets et al., 2008; Due et al., 2004). There were no differences in other dietary components between groups eliminating aspects of diet as confounding variables.
Conclusions
As the problems of obesity and its impacts on sleep and mood shape our current society, it has become necessary to turn to dietary interventions as a means to improve overall well-being. While some aspects of the study differed, it is necessary to pursue future research in this field to improve the lives of those who face these problems.

Acknowledgments
This work was funded by the Arkansas Biosciences Institute, a Student Undergraduate Research Fellowship from the Arkansas Department of Education, and Bumpers Honors College.

Literature Cited
Meet the Student-Author

I went to Farmington High School in Farmington, Arkansas and lived in Fayetteville my whole life. I am a 3-year member of the University of Arkansas Soil Judging Team. I have been 2nd in individuals overall in three consecutive Region IV soil judging competitions, while the team has placed 1st. I have participated in two national soil judging competitions, highlighted by a 2nd-place team-judged pit finish in 2023 and 6th place overall finish. I have worked as a research assistant, resident assistant, and as student manager for the Arkansas men’s basketball team. I am a Presidential Scholar, the recipient of the John W. White Outstanding Student Award, Crop, Soil and Environmental Sciences (CSES) Senior Award, and numerous departmental and collegiate scholarships. Through CSES, I have worked on multiple research projects, including a rainfall-runoff simulation experiment, a greenhouse gas emissions study in flood- and simulated-furrow-irrigated rice, an aggregate stability study, and my own Honors research, which has cultivated a love and appreciation for research. After graduation, I aspire to earn a Master’s degree from the University of Arkansas and eventually earn a Ph.D. degree in some area related to environmental science/natural resources to allow me to pursue a career as a professor. I would like to thank Dr. Brye for his gracious, unwavering mentorship throughout my entire life, and I would also like to thank Diego Della Lunga, Chandler Arel, and Morgan Brye for their assistance in the greenhouse.

Research at a Glance

- Recovering wastewater phosphorus to produce struvite could remediate ecosystems affected by excess nutrients.
- Struvite could decrease global dependence on unsustainable sources of rock-phosphate-derived fertilizer.
- Electrochemically precipitated struvite may be a viable substitute for rock-phosphate fertilizers.
Rice Biomass Response to Various Phosphorus Fertilizers in a Phosphorus-Deficient Soil Under Simulated Furrow-Irrigation

Jonathan B. Brye,* Kristofor R. Brye,† and Diego Della Lunga§

Abstract
Wastewater-recovered phosphorus (P), in the form of the mineral struvite (\(\text{MgNH}_4\text{PO}_4\cdot6\text{H}_2\text{O}\)), may provide a sustainable alternative to decreasing rock-phosphate reserves. Struvite can be generated via precipitation methods, potentially reducing the amount of P runoff to aquatic ecosystems. The objective of this greenhouse tub study was to evaluate the effects of chemically and electrochemically precipitated struvite (CPST and ECST, respectively) on aboveground plant response in a hybrid rice cultivar grown using furrow-irrigation compared to other common fertilizer-P sources [i.e., triple super phosphate (TSP) and diammonium phosphate (DAP)] using three replications of fertilizer treatment in a P-deficient silt loam (Typic Glossaqualfs). Aboveground rice dry matter (DM), aboveground DM P uptake, grain yield, and grain P uptake from CPST and ECST did not differ from DAP or TSP. However, aboveground DM P concentration was numerically largest (\(P < 0.05\)) from TSP (0.05 %), which did not differ from DAP, and was at least 2.5 times larger than that from ECST, CPST, and the unamended control (UC). Similar rice responses among struvite and other common fertilizer-P sources suggest CPST and ECST are both possible alternative fertilizer-P sources that warrant further research into struvite’s role in food production and water quality restoration and preservation.

* Jonathan Brye will be a May 2024 honors program graduate with a degree in Environmental, Soil, and Water Science.
† Kristofor Brye, the faculty mentor, is a University Professor in the Department of Crop, Soil and Environmental Sciences.
§ Diego Della Lunga is a Senior Graduate Assistant in the Department of Crop, Soil and Environmental Sciences.
Introduction

In an agronomic setting, optimal P improves systematic functions of photosynthesis, leading to healthier and more productive plants, which ultimately correlates to greater crop yields. In contrast to N and K, in moist, upland soils, P is generally highly insoluble in the soil, which leads to limited plant-available P in the soil solution (Weil and Brady, 2016). Approximately 90% of the current global P supply is mined as phosphoric or rock phosphate (RP), which is then processed to create several fertilizer-P materials. However, RP production is expected to reach a peak in the next 50 years, when Earth’s finite supply of RP will be nearly depleted (Cordell et al., 2009). One possible solution to the limited supply of mined RP is the mineral struvite (MgNH₄PO₄·6H₂O) (Omidire et al., 2020). Under the right physiochemical conditions, struvite precipitates inside wastewater treatment plant (WWTP) pipes, which is a major problem for WWTP operation on account of clogged pipes. However, when struvite-producing conditions are controlled in specialized reactors through manipulated sludge digesting processes, WWTPs can intentionally produce an abundance of struvite and prevent struvite buildup in WWTP pipes (Talboys et al., 2015).

In addition to chemical precipitation, other P-extracting technologies from wastewater are available. For example, more recently, electrochemical precipitation of struvite from synthetic wastewater has been developed and studied. Electrochemical precipitation can synthesize struvite using an electrical current applied to a solution of known N and P concentration, while magnesium (Mg) is supplied to the solution through a Mg anode that partially decays in the process to release Mg ions (Kékedy-Nagy et al., 2020). Since struvite is a P-containing mineral and there is an abundance of wastewater, struvite could be an alternative fertilizer-P source for agricultural use (Omidire et al., 2020). In addition to struvite, furrow irrigation was also utilized in the study. Furrow-irrigation is conducted by the establishment of raised beds separated by furrows that extend the length of the field between the raised beds, and furrow-irrigation has been shown to use 41% to 48% less water than conventional irrigation methods (i.e., flooding in rice cultivation) (He, 2010).

The objective of this study was to evaluate the effects of struvite (i.e., ECST and CPST) compared to several other commercially available fertilizer-P sources (i.e., DAP and TSP) on aboveground plant response to rice grown under furrow-irrigation in a P-deficient, silt loam soil. It was hypothesized that both struvite-P sources (ECST and CPST), TSP, and DAP would have similar aboveground rice dry matter but that tissue-P concentrations would differ between the two struvite-P sources themselves (ECST and CPST) due to differences in source materials, where ECST was prepared from a synthetic solution containing N and P, and CPST was generated from municipal wastewater.

Materials and Methods

The soil used in this study was a Calloway silt loam (fine-silty, mixed, active, thermic Aquic Fraglossudalfs) collected on 19 April 2021 with a shovel from the upper 10 to 15 cm from a tilled field at the University of Arkansas System Division of Agriculture’s Pine Tree Research Station near Colt in St. Francis County, Arkansas. Subsamples of air-dried soil were oven-dried at 70 °C for 48 hours, crushed, and sieved through a 2-mm mesh screen for determination of sand, silt, and clay, soil pH, and electrical conductivity (EC), soil organic matter (SOM) and total C and N, and Mehlich-3 extractable nutrients (i.e., K, P, Ca, Mg, Fe, Na, Mn, Cu, S, and Zn) (Table 1).

This study was designed to evaluate rice response to five fertilizer-P treatments: ECST, CPST, TSP, DAP, and an unamended control (UC). Treatments were arranged in a randomized complete block design on a single greenhouse bench and replicated three times for a total of 15 tubs.

Approximately 26.4 kg of sieved and air-dried soil was placed into 15 plastic tubs (51 cm wide by 67 cm long by 15 cm deep) on the same greenhouse bench and separated into three blocks, with each block containing five tubs. Tubs were seeded manually with a hybrid cultivar (Gemini 214, RiceTec) on 15 May 2021.

The first of four fertilizer applications occurred approximately 10 days after seeding (DAS), where 1 g of zinc sulfate was surface-applied to the soil surface of each tub and was watered into the soil by lightly irrigating with tap water to prevent zinc deficiency, creating a more ideal rice-growing condition. At 16 DAS, at approximately the 2 to 3 leaf stage, fertilizer-P treatments were applied manually to the soil surface of each respective tub. Each tub received 0.76 g of total P, which was equivalent to the recommended fertilizer-P rate of 29.4 kg P/ha based on the initial soil-test P concentration (Table 1; Hardke, 2021), from each fertilizer-P source (i.e., DAP, TSP, ECST, and CPST). In addition to the P, each tub received 2 g N initially, either from the fertilizer-P source, urea, or a combination of both. At 27 DAS, a second N application of 3.78 g N per tub, which was equivalent to 145.7 kg N/ha, as coated urea was surface-applied to each tub and watered into the soil by lightly irrigating with tap water. At 46 DAS, a second and final split application of 0.58 g N per tub, which was equivalent to a rate of 22.4 kg N/ha, was manually surface-applied to each tub and watered into the soil by lightly irrigating with tap water. From 11
June 2021 to 17 September 2021, all tubs were manually watered using distilled water approximately every other day.

Biomass collection took place on 25 September 2021, when the rice plants were at harvest maturity. Aboveground biomass was dried for approximately 7 days at 55 °C and weighed to determine dry matter. Rice seeds were manually stripped from the aboveground dry matter and collected to determine grain yield per tub. Subsamples of rice aboveground plant tissue were mechanically ground and sieved to < 1 mm for subsequent laboratory analyses for total N, P, and Mg. Only grain P concentration was measured. Plant nutrient uptake was determined by multiplying the vegetative dry mass and measured elemental concentrations on a plot-by-plot basis. For reporting purposes, rice yield was adjusted to 12% moisture.

Based on the randomized complete block design with three replications, a one-factor analysis of variance was conducted using PROC GLIMMIX in SAS v. 9.4 (SAS Institute, Inc., Cary, N.C.) to evaluate the effect of fertilizer-P source (i.e., DAP, TSP, CPST, ECST, and UC) on aboveground plant properties in furrow-irrigated rice. Significance was judged at $P < 0.05$. When appropriate, means were separated by least significant difference at the 0.05 level.

**Results and Discussion**

Several aboveground rice tissue properties were affected ($P < 0.05$) by fertilizer-P source (Table 2). Contrary to expectation, aboveground rice dry matter was unaffected ($P = 0.24$) by fertilizer-P source. Aboveground rice dry matter ranged from 1.31 kg/m$^2$ from ECST to 2.16 kg/m$^2$ from TSP and averaged 1.70 kg/m$^2$ overall among all fertilizer-P sources. The four fertilizer treatments behaved similarly as expected, but the fertilized treatments did not differ from the UC, which was likely because of a decrease in soil pH that caused previously immobilized P to release into the soil solution and become plant available to overcome initial soil-P deficiency (Hardke, 2021).

In contrast to aboveground dry matter, aboveground rice dry matter P concentrations differed ($P < 0.05$) among fertilizer-P sources (Table 2). Aboveground P concentration was numerically largest from TSP, which did not differ from DAP, and was at least 2.5 times greater than from the other two fertilizer-P sources, which did not differ among themselves. In addition, aboveground P concentration from the UC was similar to DAP. It is possible that solubility and in-plant translocation differences among fertilizer-P sources could have resulted in more plant-available P being released from fertilizer-P sources with larger solubilities (i.e., TSP or DAP), thus causing the aboveground dry matter P concentration to be greater from TSP and DAP than from struvite.

Similar to aboveground rice dry matter, aboveground N and Mg concentrations were unaffected ($P > 0.15$) by fertilizer-P source (Table 2). Aboveground rice dry matter N concentration ranged from 0.53% from CPST to 0.65% from TSP and averaged 0.57%, while aboveground rice dry matter Mg concentration ranged from 0.55% from the UC to 0.72% from ECST and averaged 0.65% overall among all fertilizer-P sources.

Aboveground rice dry matter N uptake ranged from 7.1 g/m$^2$ from ECST to 14.1 g/m$^2$ from TSP and averaged 9.9 g/m$^2$, while aboveground rice dry matter Mg uptake ranged from 8.5 g/m$^2$ from the UC to 13.8 g/m$^2$ from TSP and averaged 10.8 g/m$^2$ overall among all fertilizer-P sources (Table 2). Uniform N application and sufficient soil Mg concentration likely explain the similar N and Mg uptakes among fertilizer-P sources. However, contrary to expectations and in contrast to their aboveground concentrations that differed among treatments, aboveground dry matter P uptake was unaffected ($P > 0.10$) by fertilizer-P source (Table 2). Aboveground rice dry matter P uptake ranged from 0.24 g/m$^2$ from ECST to 0.90 g/m$^2$ from TSP and averaged 0.47 g/m$^2$ overall among all fertilizer-P sources (Table 2).

Similar to the results of the current study, Della Luna et al. (2021) reported that aboveground rice N uptake from conventional tillage was 7.76 g/m$^2$ in 2018 and 7.44 g/m$^2$ in 2019, and rice P uptake was 0.79 g/m$^2$ in 2018 and 0.97 g/m$^2$ in 2019, which were similar to the N and K uptakes measured in the current study (Table 2).

Similar to aboveground rice tissue properties, certain rice grain properties were unaffected ($P > 0.05$) by fertilizer-P source, while grain P and Mg concentrations differed ($P < 0.04$) among fertilizer-P sources (Table 2). A rice yield response to fertilizer-P additions was expected due to the initial low soil-test P (Table 1); however, grain yield was unaffected ($P = 0.44$) by fertilizer-P source, which ranged from 1.11 kg/m$^2$ from DAP to 1.47 kg/m$^2$ from TSP and averaged 1.26 kg/m$^2$ overall among all fertilizer-P sources (Table 2). Similar to aboveground dry matter, the four fertilizer treatments behaved similarly as expected, but grain yield from the fertilized treatments was not greater than from the UC, which, similar to aboveground dry matter, was likely because of a decreased soil pH that released additional P over the course of the growing season.

In contrast to grain yield, grain P and Mg concentrations from TSP, DAP, ECST, and CPST, which did not differ, were at least 1.2 times greater than the UC (Table 2). It is unclear why Mg concentration differed between the fertilized treatments and the UC considering there was likely adequate initial soil Mg among all treatments, but it is
possible that there was a differential interaction between the soil Mg and the fertilizer-P sources during plant Mg uptake. Contrary to the current study, Omidire et al. (2022a) reported that grain P and Mg concentrations in a flood-irrigated, pure-line cultivar were unaffected by fertilizer-P source (i.e., TSP, DAP, ECST, CPST, and UC). In contrast to the current study, a 2-year field study evaluating P fertilizers (i.e., ECST, CPST, monoammonium phosphate, DAP, TSP, and rock phosphate) in corn on a silt loam (Aquic Fraglossudalfs) in eastern Arkansas reported that kernel P and Mg concentrations were unaffected (Omidire et al., 2022b).

Contrary to expectations and in contrast to their grain concentrations that differed among treatments, grain P uptake was unaffected ($P > 0.09$) by fertilizer-P source (Table 2). Grain P uptake ranged from 2.4 g/m$^2$ from the UC to 3.9 g/m$^2$ from TSP and averaged 3.2 g/m$^2$ among fertilizer-P sources.

**Conclusions**

This study evaluated the effects of two struvite materials, ECST and CPST, on the aboveground plant response of a hybrid rice cultivar grown in the greenhouse in a P-deficient, silt-loam soil under simulated furrow-irrigation compared to other common fertilizer-P sources. As hypothesized, both ECST and CPST treatments produced a similar rice response amongst the struvite treatments and the RP-derived fertilizers, but contrary to the hypothesis, tissue-P concentration was similar among struvite treatments. Based on the results of this greenhouse study, it can be concluded that struvite, namely ECST, is a viable fertilizer-P source that could be used as an alternative to RP-derived fertilizers for simulated furrow-irrigated rice production in a P-deficient, silt-loam soil.

**Acknowledgments**

This research was partially supported by a grant from USDA-NIFA-AFRI Water for Food Production Systems program (Project Number 2018-68011-28691). Greenhouse assistance was provided by Diego Della Lunga, Chandler Arel, and Morgan Brye who are gratefully acknowledged.

**Literature Cited**


Table 1. Summary of initial physical and chemical property means (n = 5) and standard errors (SE) for the soil used in the greenhouse experiment.

<table>
<thead>
<tr>
<th>Soil Property</th>
<th>Mean (± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sand (g/g)</td>
<td>0.09 (&lt;0.01)</td>
</tr>
<tr>
<td>Silt (g/g)</td>
<td>0.79 (&lt;0.01)</td>
</tr>
<tr>
<td>Clay (g/g)</td>
<td>0.12 (&lt;0.01)</td>
</tr>
<tr>
<td>Electrical conductivity (dS/m)</td>
<td>0.167 (&lt;0.01)</td>
</tr>
<tr>
<td>pH</td>
<td>7.5 (0.01)</td>
</tr>
<tr>
<td>Extractable soil nutrients (mg/kg)</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>11.4 (0.1)</td>
</tr>
<tr>
<td>K</td>
<td>46.1 (0.9)</td>
</tr>
<tr>
<td>Ca</td>
<td>2005 (4.2)</td>
</tr>
<tr>
<td>Mg</td>
<td>276.3 (2.3)</td>
</tr>
<tr>
<td>S</td>
<td>11.9 (0.4)</td>
</tr>
<tr>
<td>Na</td>
<td>29.8 (0.6)</td>
</tr>
<tr>
<td>Mn</td>
<td>244.3 (5.1)</td>
</tr>
<tr>
<td>Fe</td>
<td>303.8 (7.8)</td>
</tr>
<tr>
<td>Cu</td>
<td>1.6 (&lt;0.1)</td>
</tr>
<tr>
<td>Zn</td>
<td>2.5 (0.1)</td>
</tr>
<tr>
<td>Soil organic matter (g/kg)</td>
<td>25.7 (0.2)</td>
</tr>
<tr>
<td>Total C (g/kg)</td>
<td>11.4 (0.2)</td>
</tr>
<tr>
<td>Total N (g/kg)</td>
<td>1.1 (&lt;0.1)</td>
</tr>
<tr>
<td>C:N ratio</td>
<td>10.0 (0.1)</td>
</tr>
</tbody>
</table>

Table 2. Analysis of variance summary of the effect of fertilizer-phosphorus treatment [i.e., electrochemically precipitated struvite (ECST), chemically precipitated struvite (CPST), diammonium phosphate (DAP), triple superphosphate (TSP), and unamended control (UC)] on aboveground dry matter, aboveground dry matter elemental concentrations and uptake, grain yield, and grain P uptake for rice grown in the greenhouse under simulated furrow-irrigated conditions.

<table>
<thead>
<tr>
<th>Plant Property</th>
<th>P-value</th>
<th>ECST</th>
<th>CPST</th>
<th>DAP</th>
<th>TSP</th>
<th>UC</th>
<th>Overall Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter (kg/m²)</td>
<td>0.24</td>
<td>1.31</td>
<td>1.90</td>
<td>1.57</td>
<td>2.16</td>
<td>1.54</td>
<td>1.70</td>
</tr>
<tr>
<td>Dry matter concentration</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N (%)</td>
<td>0.38</td>
<td>0.54</td>
<td>0.53</td>
<td>0.60</td>
<td>0.65</td>
<td>0.55</td>
<td>0.57</td>
</tr>
<tr>
<td>P (%)</td>
<td>0.03</td>
<td>0.017 c</td>
<td>0.016 c</td>
<td>0.036 ab</td>
<td>0.047 a</td>
<td>0.020 bc</td>
<td>--</td>
</tr>
<tr>
<td>Mg (%)</td>
<td>0.15</td>
<td>0.72</td>
<td>0.65</td>
<td>0.68</td>
<td>0.64</td>
<td>0.55</td>
<td>0.65</td>
</tr>
<tr>
<td>Dry matter uptake</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N (g/m²)</td>
<td>0.24</td>
<td>7.08</td>
<td>10.02</td>
<td>9.68</td>
<td>14.06</td>
<td>8.58</td>
<td>9.88</td>
</tr>
<tr>
<td>P (g/m²)</td>
<td>0.10</td>
<td>0.24</td>
<td>0.34</td>
<td>0.58</td>
<td>0.90</td>
<td>0.27</td>
<td>0.47</td>
</tr>
<tr>
<td>Mg (g/m²)</td>
<td>0.10</td>
<td>9.25</td>
<td>11.95</td>
<td>10.59</td>
<td>13.83</td>
<td>8.53</td>
<td>10.8</td>
</tr>
<tr>
<td>Grain yield (kg/m²)</td>
<td>0.44</td>
<td>1.13</td>
<td>1.42</td>
<td>1.11</td>
<td>1.47</td>
<td>1.19</td>
<td>1.26</td>
</tr>
<tr>
<td>Grain P concentration (%)</td>
<td>0.02</td>
<td>0.26 a</td>
<td>0.26 a</td>
<td>0.27 a</td>
<td>0.27 a</td>
<td>0.20 b</td>
<td>--</td>
</tr>
<tr>
<td>Grain Mg concentration (%)</td>
<td>0.04</td>
<td>0.11 a</td>
<td>0.11 a</td>
<td>0.12 a</td>
<td>0.12 a</td>
<td>0.09 b</td>
<td>--</td>
</tr>
<tr>
<td>Grain P uptake (g/m²)</td>
<td>0.10</td>
<td>2.98</td>
<td>3.72</td>
<td>3.05</td>
<td>3.87</td>
<td>2.39</td>
<td>3.20</td>
</tr>
</tbody>
</table>

† Means in a row with different letters are different at P < 0.05.
Temporal, Phenotypic, and Quantitative Characterization of Thyroid Infiltrating Mononuclear Cells During Development of Spontaneous Autoimmune Thyroiditis in Obese Strain Chickens

Meet the Student-Author

Katelyn Clark

I am a Summa Cum Laude May 2023 graduate with a B.S. in Poultry Science and B.A. in Spanish. Prior to attending the University of Arkansas, I went to Fayetteville High School. I am currently interning with Cobb Vantress in their lab and have previously worked at a veterinary clinic in high school and college. In high school, I was also part of the Veterinary Science team for FFA, participated in Beef Quiz Bowl, and took several animal science courses. These activities ultimately inspired me to major in Poultry Science. I was also part of the Health Occupations Students of America Veterinary Science team and competed at their International Leadership Conference before serving as a mentor for the veterinary science team at Fayetteville High School while in college the next year. Having a keen interest in animal health and welfare, I was very interested in immunology, which drew me to my honors thesis topic. I have enjoyed my time at the University of Arkansas and within the honors college and am extremely grateful for the opportunities that both have afforded me. I would like to thank Dr. Gisela F. Erf and Chrysta Beck for their continued guidance and my committee members, Drs. Sara K. Orlowski and Adnan Alrubaye, for their assistance. I would also like to thank Jossie Santamaria for his assistance in the lab and my friends and family who have supported me throughout this chapter of my life.

Research at a Glance

- Thyroids were collected from Obese strain chickens prone to spontaneously develop an autoimmune disease affecting the thyroid glands mimicking Hashimoto’s thyroiditis in humans.

- Thyroid samples were immunochemically stained to identify different types of mononuclear immune cells infiltrating the thyroid. The samples could then be viewed under a microscope to estimate the proportion of the gland tissue occupied by each type of immune cell type.

- Immune cell infiltration was first observed at 7 days of age, and from 3 weeks onwards, infiltration was nearly complete across most samples. T cells and B cells were the most numerous immune cells present.

Katelyn examining the thyroid tissues under a microscope to evaluate the relative amounts of various immune cells present based on brown staining.

Photo credit: Russell Cothren
Temporal, Phenotypic, and Quantitative Characterization of Thyroid Infiltrating Mononuclear Cells During Development of Spontaneous Autoimmune Thyroiditis in Obese Strain Chickens

Katelyn M. Clark,* Chrysta N. Beck,† and Gisela F. Erf§

Abstract

The Obese strain (OS) of chickens spontaneously develops autoimmune thyroiditis (SAT) and is a well-established biomedical model for Hashimoto’s thyroiditis in humans. Both conditions are characterized by the infiltration of thyroid glands with mononuclear immune cells resulting in the destruction of thyroid tissue and impairment of the thyroid’s endocrinological functions. Past studies described immune cell infiltration in thyroids of the OS chickens, but the time-course, cell composition, and relative amounts of the various immune cells infiltrating the thyroids have not been well defined. In this project, frozen and stored thyroid glands that were previously collected at 1, 4, 7, 14, 21, 28, 35, and 42 days of age (n = 4 to 5 OS birds/age) were used. Frozen thyroid sections (8-μm thick) were prepared and used in an indirect immunohistochemical staining procedure to identify macrophages, B cells, T cells, T helper cells, cytotoxic T cells, γδ T cells, and MHC II-expressing cells. Stained sections were evaluated by microscopy, and the percentage of tissue area occupied by various cell types was determined. Thyroid infiltration was first observed at 7 days of age, and immune cells occupied the entire tissue in most samples from 3 weeks onwards. Macrophages were the first cells to infiltrate, but T cells dominated the response. MHC II expression reached very high levels by 14 days and remained at nearly 100% thereafter. This study provided new insights regarding the participating immune cells and the chronological order of their infiltration into thyroid glands during SAT development in OS chickens.

* Katelyn Clark is a May 2023 honors program graduate from the Department of Poultry Science.
† Chrysta Beck, Graduate Assistant, Department of Poultry Science.
§ Gisela Erf, the faculty mentor, is a professor in the Department of Poultry Science.
Introduction

Autoimmune diseases have a significant impact on quality of life as they often require long-term management. Hashimoto’s thyroiditis is one of the most common autoimmune diseases in the United States, affecting one to two percent of the population (MedlinePlus, 2020). Hashimoto’s thyroiditis is characterized by the infiltration of the thyroid with a variety of immune cells that results in damage and destruction of functional thyroid tissue and consequently hypothyroidism. Infiltrating T cells were shown to have cytotoxicity towards thyroid antigens resulting in apoptosis of thyroid follicular cells, while B cells produce specific autoantibodies to thyroid-associated molecules (Ajjaj and Weetman, 2015).

High levels of thyroid-stimulating hormone (TSH), low levels of thyroxine (T4), and the presence of anti-thyroid peroxidase and anti-thyroglobulin antibodies are the most critical diagnostic criteria, but Hashimoto’s thyroiditis is often not diagnosed until there is considerable damage to the thyroid (Mincer and Jialal, 2022). Once the thyroid is damaged, a broad range of symptoms can occur, such as weight gain, cold intolerance, goiter, depression, joint pain, memory lapses, and fatigue (Mayo Clinic, 2020). Because Hashimoto’s thyroiditis goes undiagnosed until late into the disease progression, studying the progression of the autoimmune disease in humans is difficult. As a result, biomedical animal models can play an important role in research focused on the development and progression of autoimmune destruction in the thyroid.

The Obese strain (OS) of chicken housed at the University of Arkansas System Division of Agriculture in Fayetteville, Arkansas, develops spontaneous autoimmune thyroiditis (SAT). The OS line was developed by Randall Cole at Cornell University after he noticed odd physical characteristics in a few of the pullets in the Cornell strain (CS) in 1955 that are now associated with OS chickens: the smaller stature, long and silky feathers, increased subcutaneous fat accumulation, and diminished reproductive development (Cole, 1966). The SAT in OS chickens mimics Hashimoto’s thyroiditis and provides an excellent opportunity for investigation into the development of this organ-specific autoimmune disease, especially as SAT develops rapidly with mononuclear immune cell infiltration of thyroid glands beginning within the first week of life (Dietrich et al., 1997). This early, spontaneous, and predictable onset of SAT makes this animal model particularly suitable to study the immunopathology before onset and during the development and progression of the disease (Erf, 2021).

Autoimmune diseases are derived from a loss of self-tolerance of T- and/or B-lymphocytes that results in a specific immune response to self-antigens. In organ-specific autoimmunity, like SAT, other mononuclear cells, such as macrophages, may also play a role in tissue destruction (Janeway et al., 2001). Characterizing the infiltrating mononuclear cells (lymphocytes and macrophages) present both before and during thyroid destruction in OS chickens could provide information on how Hashimoto’s disease is initiated and progresses. This study aims to characterize and quantify the mononuclear cells, specifically B cells, various T cell subpopulations, and macrophages, which infiltrate thyroid glands in OS chickens, as well as establish a timeline of mononuclear cell infiltration within the first six weeks of the OS chicken’s life.

Materials and Methods

The animal experiment and tissue collection for this study were previously conducted by Dr. G.F. Erf, Department of Poultry Science, University of Arkansas System Division of Agriculture, and her team. A total of 36 birds from the OS line were used for the study. The birds were raised in floor pens under conventional husbandry practices at the Poultry Health Lab. All procedures involving the experimental animals were approved by the University of Arkansas System Division of Agriculture, Institutional Animal Care and Use Committee (IACUC; protocol 21077).

At 0, 4, 7, and 14 days of age, 5 birds were euthanized via CO₂ inhalation, and their thyroid glands were collected. After 14 days, 4 birds were euthanized for thyroid collection each week until the birds were 6 weeks of age. The thyroid glands collected at each time point were placed in plastic histology molds, covered in OCT freezing medium, snap frozen in liquid nitrogen, and stored at -80 °C, with the thyroid glands from the left side being in one mold and the thyroid glands from the right side in another mold.

For this project, an indirect immunohistochemical staining procedure was used to identify various immune cells and molecules in the thyroid glands, following the procedures described by Sorrick et al. (2022). Specifically, the thyroid glands were cut with a cryostat at -24 °C into 8-μm thick sections, individual sections placed on poly-L-lysine coated glass microscope slides, and sections fixed in acetone for 5 minutes. To prevent non-specific binding of the reagents used for the immunostaining to the cells, the sections were incubated overnight with 10% horse serum in phosphate-buffered saline (PBS) in a humidifying chamber. After incubation and each subsequent incubation step (Table 1), sections were washed with PBS. Next, the sections were incubated for 30 minutes with primary mouse anti-chicken (mac) monoclonal antibodies, which included antibodies specific to the cell surface molecules: CD3 (pan T cell marker), CD4 (T helper cells), CD8 (cytotoxic T cells) and γδ T cell receptor (TCR), Bu-I (B cells), KUL-01 (macrophages), and MHC II (appears on...
a variety of cells and functions as an antigen-presenting molecule). All primary mac-monoclonal antibodies were IgG1. Hence, to check for the presence of non-specific binding of the reagents used for the immunostaining of the cells, a mouse IgG1, monoclonal antibody with irrelevant specificity (isotype control) was used instead of the primary specific antibody on a thyroid section. All primary antibodies and the isotype control were purchased from Southern Biotech, Birmingham, Alabama.

To detect binding of the primary antibodies, sections were then incubated for 30 minutes with the secondary antibody, a biotinylated horse anti-mouse (ham) IgG antibody that binds to the primary mac-antibodies (Vector Laboratories, Inc., Burlington, California). The tissue sections were then incubated with ABC reagent, consisting of avidin, which was preincubated with biotin conjugated with horse radish peroxidase (HRP) enzyme following manufacturer instructions (Vector Laboratories). Following the 30-minute incubation with ABC reagent, diaminobenzidine tetrahydrochloride (DAB) that was charged with peroxide was then added to the sections to serve as a substrate for the HRP enzyme. As a result of the enzyme-substrate reaction, a brown product was formed that precipitated at the site of formation; the brown precipitate visually identified the cell with the cell surface molecule the specific primary antibodies bound to, hence identifying a cell based on its unique cell-surface molecule. When the brown precipitate developed, the sections had a final wash with PBS and were counterstained with Methyl Green nuclear stain. The sections were then sealed with a glass coverslip and VectaMount mounting medium (Vector Laboratories).

Tissues were viewed at 40x magnification using a bright field Olympus BX50 microscope to determine the location and proportion of the types of immune cells/cell surface molecules identified by immunohistochemical staining. Tissue images were captured via a cool SNAP camera connected to a computer with Image-Pro Plus software. Images of each section were visually evaluated for the extent of brown precipitation for each cell-type/marker. The areas of brown precipitation and, in the case of overall infiltration, the dense areas of green nuclear staining were assessed subjectively as the portion (%) of the thyroid section with brown or green stain, respectively, by the same evaluator. Proportions were averaged for tissues requiring evaluation of more than one image. One-way analysis of variance was used for statistical analysis to determine the effect of age for each cell type/marker. In the case of a significant age effect ($P \leq 0.05$), Fisher’s least significant difference multiple means comparisons tests were conducted to determine differences ($P \leq 0.05$) between means at each age.

### Results and Discussion

There was no brown precipitation on the thyroid sections stained with the isotype control, and hence no non-specific binding of any of the reagents used. As only green counterstain was visible on these sections, they were used to evaluate total mononuclear cell infiltration. There was an effect of age for infiltration ($P < 0.001$; Figs. 1–3). Although there was variation in the severity of infiltration within each time point, some general trends were observed from the day of hatch to 42 days of age (Fig. 1). There was no noticeable infiltration until 7 days, when there was mild infiltration at 13 ± 7.8%. The incidence and severity of infiltration increased until 28 days, when nearly the entire thyroid section consisted of mononuclear cells instead of

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<sup>a</sup> PBS = phosphate buffered saline; HS = horse serum; Ab = antibody.

<sup>b</sup> All primary antibodies were mouse anti-chicken (mac) IgG1 isotype.

<sup>c</sup> ham = horse anti-mouse IgG antibody, conjugated with biotin.

<sup>d</sup> ABC = avidin-biotin complex, a pre-reacted mixture of avidin and peroxidase labeled biotin.

<sup>e</sup> DAB = 3,3′-Diaminobenzidine, substrate for the peroxidase enzyme on ABC that results in formation of a brown product that precipitates at the site of formation.

<sup>f</sup> Isotype control = non-specific binding control, mouse IgG1 antibody with irrelevant specificity.
Fig. 1. Thyroid infiltrating immune cells in thyroids from Obese strain chickens during the development of spontaneous autoimmune thyroiditis. Frozen sections of thyroids collected at various ages from day of hatch until 42 days of age were stained via indirect immunohistochemistry using mouse anti-chicken primary antibodies specific for T cells (CD3), B cells (Bu-1), T cell subsets (CD4, CD8, and γδ T cell receptor), macrophages (KUL01) and MHC II expression. Data are mean ± SEM of the estimated percentage of thyroid tissue occupied by various cell types. n = 3 to 5 thyroids per time point; a-c: means without a common letter are different at P ≤ 0.05.
thyroid follicles. Similar observations of nearly complete replacement of thyroid tissue by infiltrating mononuclear cells were made in thyroids collected at 35 and 42 days (Fig. 1). At 14 days, the first observation of nearly complete infiltration was made (Figs. 1 and 2), but other tissues had much lower severity with a third or less of the tissue occupied by mononuclear cells (Fig. 3).

T lymphocytes were the most prominent leukocyte within the thyroid tissue, and there was an effect of age ($P = 0.004$). T cell proportions in the thyroids followed a similar trend to overall infiltration, with the first noticeable appearance at 7 days with an average of $10 \pm 6.5\%$, but the proportion of T lymphocytes stabilized around 21 days at $47.0 \pm 8.5\%$ reaching maximal levels at 35 and 42 days of $55 \pm 4.6\%$ and $55 \pm 10.4\%$, respectively (Fig. 1). Like T lymphocytes, B lymphocytes also first appeared on day 7, but occupied only 4% of the thyroid section area (Figs. 1 and 3). B cell levels then gradually increased, reaching their highest proportion at $38 \pm 12.8\%$ at 42 days (Fig. 1). The CD4+ and CD8+ T cells were present in relatively equal proportions up to 14 days of age (Fig. 1). The CD4+ to CD8+ ratio within this two-week period ranged from 0.76 to 1. From 7 days to 42 days, the levels of CD4+ T cells ranged from $8.3 \pm 5.5\%$ up to $48\% \pm 5.2\%$ at 42 days (age, $P < 0.001$), whereas the CD8+ T cell population fluctuated between $11 \pm 9.7\%$ and $26 \pm 2.1\%$ with no differences (age, $P = 0.284$), resulting in a CD4+ to CD8+ T cell ratio (CD4:CD8 ratio) of $1.89 \pm 0.25$ at 42 days. While the average proportion of $\gamma \delta$ T cells never exceeded 5% of the area of the thyroid tissue, the proportions of this cell type also increased with time (age, $P = 0.002$) (Fig. 1).

Macrophages also were present in small amounts. The most notable time points in terms of the presence of macrophages occurred at 35 and 42 days (age, $P = 0.017$), but even

Fig. 2. Chicken thyroid sections with and without immune cell infiltration. a-b) Methyl Green-stained frozen sections of normal thyroids from a) a 4-day-old autoimmune thyroiditis-prone Obese Strain (OS) chick and b) a 6-week-old White Leghorn control chicken showing normal thyroid tissue without immune cell infiltration. c-d) Extensive lymphocyte infiltration of a thyroid gland from a 14-day-old OS chicken; T cells (c) and B cells (d) were identified by indirect immunohistochemical staining using chicken-CD3 and Bu-1 specific primary mouse monoclonal antibodies, respectively. Note: nearly all the thyroid tissue is occupied by T and B cells. Pictures were taken at 40x magnification on a bright field Olympus BX50 microscope equipped with a coolSNAP™ camera.
at the maximal point at 42 days, macrophages were only present at 6.8 ± 2.2%. It should be noted, however, that macrophages were present at a small proportion (1 to 2%) as early as the day of hatch and 4 days, unlike other leukocytes examined (Fig. 1).

Kite et al. (1969) reported a predominance of large mononuclear cells based on conventional histology. While the time course and extent of mononuclear cell infiltration of OS thyroids in the present study agreed with Kite et al. (1969), specific identification of macrophages based on the expression of macrophage cell surface molecule KUL-01 revealed a bimodal pattern of macrophage infiltration. In the current study, we found low but early presence of macrophages, with relative proportions dropping during the major lymphocyte infiltration phase and increasing again when tissue destruction was extensive. Our observations regarding macrophages also agree with Hala et al. (1996), who concluded that macrophages were one of the first cells to infiltrate the thyroid, followed shortly thereafter by B- and T-lymphocytes. Together these observations point towards the role of the macrophages in the initiation of the mononuclear cell infiltration, as well as in the later removal of dying and dead cells during the autoimmune destruction of the thyroid tissue (Abbas et al., 2018).

As described by Hala et al. (1996) and observed in the current study, macrophage infiltration was followed

![Images of CD4+ T cells, CD8+ T cells, and B cells infiltration in thyroids](image)

**Fig. 3.** Images of CD4+ T cell-, CD8+ T cell-, and B cell-infiltration in thyroids during the development of spontaneous autoimmune thyroiditis in Obese Strain chickens at 7- and 21-days of age. Frozen sections of thyroids collected from Obese Strain chickens at various ages from the day of hatch until 6 weeks of age were stained using an indirect immunohistochemical staining procedure utilizing a mouse anti-chicken primary antibody specific for CD4, CD8, and Bu-1 to identify CD4+ T cells, CD8+ T cells and B cells, respectively. Pictures were taken at 40x magnification on a bright field Olympus BX50 microscope equipped with a coolSNAP™ camera.
shortly thereafter by B- and T-lymphocytes, and these small mononuclear cells appeared to be the primary constituents of the thyroid infiltrating cells, with more than 2-fold higher proportions of T cells than B cells at specific time points. As expected, B cells were organized into tightly packed follicles, surrounded by T cells, which also were often observed as aggregates. B cell follicles, described as germinal centers by Hala et al. (1996) and Wick et al. (2006), contain B cells at various stages of activation and differentiation, including proliferating cells, B cells with different isotypes of B cell receptors, and antibody-producing B cells, aka plasma cells. Unfortunately, we did not have markers to identify the different populations of B cells. However, proliferating B cells and plasma cells would morphologically be described as large mononuclear cells, as would proliferating T cells, which may be present in the T cell aggregates. These lymphocyte populations could have been part of the large mononuclear cells described in Kite et al. (1969).

MHC II is found on a variety of cells, and many of the tissues were populated quite densely with MHC II positive cells. By 21 days (age, $P = 0.002$), almost the entire tissue was covered with brown precipitate indicating MHC II expression, which remained the case until 42 days of age (Fig. 1).

Considering that T helper cells need antigen-presentation in association with MHC II on antigen-presenting cells, the extensive expression of MHC II in affected thyroids may be important in their inflammatory activities. However, the extent of MHC II expression cannot be explained by the presence of antigen-presenting cells (macrophages, dendritic cells, and B cells alone) and is likely a reflection of interferon-$\gamma$ production by infiltrating CD4+ T cells, which in chronic inflammation causes expression of MHC II on non-immune cells as well. Moreover, activated chicken T cells, like activated human T cells (but not mouse T cells), may also express MHC II (Abbas et al., 2018). Overall, the extensive expression of MHC II with increasing mononuclear cell infiltration is likely a reflection of the chronic inflammatory activity taking place in this thyroid-specific autoimmune response.

Conclusions

The current study revealed the time course and profiles of participating immune cells during the development of SAT in the Obese strain chicken model. Immune cell infiltration of the thyroid started within two weeks of life and progressed to complete tissue infiltration by 6 weeks; however, little is known regarding the functional activities of the thyroid infiltrating immune cells (e.g., cytokine production, cytotoxicity). Moreover, it is not clear what is happening to hormone production in the thyroid during this same time period and at what point the destruction of the thyroid begins to impact thyroid hormone concentrations. However, the current study laid the foundation to address these questions in future studies to elucidate what causes the onset and drives the progression of thyroid infiltration and tissue destruction and consequently, the development of hypothyroidism.

Acknowledgments

This project was made possible by the Tyson Endowed Professorship in Avian Immunology (G. F. Erf).

Literature Cited


In May of 2023, I graduated Summa Cum Laude with a degree in Animal Science and Spanish. I am from Greenwood, Arkansas, where I attended Greenwood High School. During my time at the University of Arkansas, I was a member of the Block and Bridle club, where I served as secretary and president, a member of the Meat Science Quiz Bowl Team, a member of the Animal Science Quadrathlon Team, and an Animal Science REPS member. I was also able to gain hands-on experience working in the Food and Poultry Science labs. Outside of class, I taught the adult Sunday school class at my home church and often volunteered with Apple Seeds Teaching Farm. I will be attending Kansas State University in the fall of 2023 to work on a master's degree in Meat Science. I intend to continue my education and pursue a career in education and research at the university level. I have always been interested in the food industry, but I had no idea of the scope of its impact until I began working on this project. It has been an honor to work with my honors mentor, Dr. Kelly Vierck, and her graduate students, Lizzi Neal and Katie Boatright, as well as Dr. Janeal Yancey and Dr. Derico Setyabrata. Without them, I would not have found my passion for meat science and a career I love building each day.

Meet the Student-Author

Jordan Looper

Jordan using the homogenizer to blend samples as a step in the thiobarbituric acid reactive substances assay.

Determining the Effectiveness of Rosemary Essential Oil on the Shelf Life of Ground Beef Under Different Lighting Conditions

Research at a Glance

- Color is one of the most important factors consumers use in determining product desirability.
- Antioxidants are used to help extend the shelf life of a variety of products, including produce and meat products.
- Lighting intensity and antioxidant manipulation are two possible means of increasing the shelf life of ground beef.
Determining the Effectiveness of Rosemary Essential Oil on the Shelf Life of Ground Beef Under Different Lighting Conditions

Jordan T. Looper* and Kelly R. Vierck†

Abstract
This study determined the effectiveness of rosemary extract on the shelf life of ground beef patties under different retail display conditions. Ground beef patties were produced from an 85%:15% blend (lean:fat). Patties were formed from batches of control or amended with rosemary extract. Patties were individually packaged using overwrap. Groups were assigned into one of two lighting groups (3000K and 3500K). Patties were placed in a simulated retail display for 5 d under continuous lighting and rotated once a day. Lipid oxidation and color samples were taken each day. Relating to lipid oxidation, there was no three-way interaction between display day, antioxidant, and light intensity ($P > 0.05$). There was an interaction observed between antioxidant and day ($P < 0.0001$). Relating to color spectrometry, L* values presented an interaction between antioxidant and lighting intensity ($P = 0.0029$). A two-way interaction between day and antioxidant ($P = 0.0003$) was also shown in a* values and b* values ($P = 0.0008$). Chroma values displayed an interaction between antioxidant and day ($P = 0.0008$). The hue data concluded similar results ($P = 0.0008$); there was an interaction observed between antioxidant and the reduction of retail display days. These data suggest that antioxidants reduce lipid oxidation regardless of light temperature. Antioxidants can still be used to extend shelf life and improve color stability in ground products.

* Jordan Looper is a May 2023 honors program graduate with a major in Animal Science.
† Kelly R. Vierck, the faculty mentor, is an Assistant Professor in the Department of Animal Science.
Introduction

The use of natural alternatives as food additives to improve taste, texture, appearance, and shelf-life has become increasingly popular in recent years, both from a consumer and food industry perspective. Research into the topic of bacterial growth, when exposed to natural oils, has been occurring since the 1970s, and with the growth in popularity and availability of natural oils in the last decade, inquiries into the role and function of these oils in the food industry have increased. Essential oils and naturally growing plants have been shown to act similarly to some current synthetic products that extend shelf-life when used with fresh produce but provide negative olfactory responses due to unique odors and tastes associated with these oils (Rodriguez et al., 2015). These plant-based oils, however, are limited by these flavor compounds resulting in certain antioxidants being better suited for specific food groups than others, and their synthetic counterparts provide a more cost-efficient and more stable product that consistently performs higher (Pokorný, 2007). Studies have shown the potential for synthetic antioxidants to be harmful to health, and although the numbers are extremely low, adverse reactions to these additives are possible; however, natural alternatives cannot be determined to be entirely safe either (Randhawa and Bahna, 2009; Pokorný, 2007). In recent years, research performed during animal processing has been underdeveloped, yet reports on the effects of oils utilized during nutritional supplementation of live animals and during processing practices have grown significantly. This form of shelf-life extension could potentially provide a multitude of benefits for consumers, including decreased prevalence of foodborne illness, alternatives to synthetic antioxidants, and the use of a cleaner label for consumers. While the driving force for more natural alternatives primarily appears to stem directly from the consumer side, potential outcomes of research within the beef industry, or other food and beverage sectors, could provide novel and impactful results for a product that meets consumer needs and desires (McDonnell et al. 2013).

The goals of this study included determining the effect of essential oils on ground beef storage in conjunction with alterations in lighting intensity. The causality that was expected was that an extension of shelf life for ground beef treated with essential oils in combination with a lower light intensity would result after a common period in retail display. Through previously conducted research, the positive correlation between shelf life and essential oil, as well as between shelf life and lower lighting intensity, implies the possibility of synergism when the two are combined.

Materials and Methods

Ground beef was purchased locally with an 85% lean and 15% fat ratio, fine ground through a 0.953 cm plate, and separated into 151.2 g patties (n = 64) using the Hollymatic Super Patty Machine. During grinding, Kalsec® Oleoresin Rosemary, Herbalox® Brand XT-25 was added with a concentration of 0.20% (Keokamerd et al., 2008) to half the beef. Patties were assigned randomly to one of two treatments, a control group and a group treated with essential oil. Patties were individually packaged in foam trays with an oxygen-permeable polyvinyl wrap. Patties, within antioxidant treatment, were assigned randomly to two different lighting temperatures (3000 K or 3500 K) in retail placement. Six batches were created with three antioxidant batches per antioxidant treatment. A completely randomized split-plot design was used. One batch served as the whole plot, and lighting served as a whole plot. Ground patties were subjected to a simulated retail display for five days under continuous light-emitting diode (LED) lighting at 4 °C. Patties were rotated once each day following thiobarbituric acid reactive substances (TBARS) and color data collection. These patties were rotated randomly within the shelving of the display case, moving internally in the shelves as well as levels in the case. Ground beef patties were displayed in the simulated retail case for five days, with the control and antioxidant treatments assorted randomly throughout the two separate lighting cases.

During each day of retail display, the instrumental color of patties was determined using the Hunter Lab MiniScan EZ spectrophotometer. A randomly generated list of the patties was used to determine the patties used for each day of color. The L*, a*, and b* values were taken to determine lightness, redness, and yellowness, respectively; the hue values were taken to measure the vividness; and the chroma values were taken to determine saturation (King et al., 2023). Hue was calculated by taking the arctangent of the b* values divided by the a* values, while chroma was calculated by squaring both a* and b* values and taking the square root of the sum, according to the American Meat Science Association Guidelines (King et al., 2023). Three measurements were taken per patty and averaged together to provide an overall color measurement for each patty. Subsets of patties were frozen for days 1–5 following placement in retail display.

Each sample was then thawed for 10 to 12 hours to 5 °C, placed in liquid nitrogen, and powdered using a Nutribullet blender. A randomly generated list of the patties was used to determine the patty sample collected for analysis. A 10-g sample was weighed into a 50-mL conical tube, and TBARS, an assay measuring malondialdehyde as a representation of lipid oxidation, were analyzed through the modified procedure of Buege and Aust (1978) as described by Luque et al. (2011). A standard curve for the assay was run for each day of testing. Samples were blended with 30 mL of deionized water and then centrifuged. Two mL of the supernatant was removed and added to a 50-mL centrifuge tube with the...
trichloroacetic acid reagent and butylated hydroxyanisole. Samples were heated, cooled, and centrifuged. Two 1-mL samples were added to a 48-well plate and then analyzed.

Data were analyzed as a split-split plot design, with batch serving as the whole plot and patty serving as the subplot. Fixed effects in the model were lighting temperature, antioxidant treatment, and day of display. The Kenward-Rogers adjustment was used with all analyses. Statistical differences were considered significant at $\alpha \leq 0.05$.

**Results and Discussion**

There was no three-way interaction between display day, antioxidant, and light temperature ($P > 0.05$), as well as no interactions between display day and light temperature ($P > 0.05$), and antioxidant and lighting temperature ($P > 0.05$) on TBARS. No effect of lighting temperature was observed ($P > 0.05$). There was an interaction observed between antioxidant and day ($P < 0.0001$) (Fig. 1). Overall, a larger separation between control and antioxidant was shown through each progressive day of display, expressing a linear response of lipid oxidation in the control group, while the treated antioxidant group remained relatively consistent. Additionally, a main effect of antioxidant ($P < 0.05$) and display day ($P < 0.05$) were observed. The patties with the antioxidant treatment expressed lower lipid oxidation than the control patties, regardless of lighting intensity ($P < 0.05$). Furthermore, a reduction in display day yielded a net reduction in lipid oxidation, regardless of lighting temperature or antioxidant supplementation ($P < 0.05$).

There were no three-way interactions observed for any of the color traits evaluated ($P < 0.05$). There was no interaction between day and antioxidant presented in L* values ($P > 0.05$). There was an interaction between antioxidant and light ($P = 0.0029$), indicating that lightness value increases as lighting intensity increases (Fig. 2). When antioxidant treatment was included, there was no difference between the groups at 3500 K; however, at 3000 K, antioxidants showed an increase in lightness. There was a main effect of day ($P < 0.0001$) with a predominate linear decline in L* as day progressed, with the exception of day 0, implying that the addition of the oleoresin antioxidant could have played a role in the lower lightness value.

There was also no interaction between day and lighting intensity ($P > 0.05$) or between lighting intensity and antioxidant ($P > 0.05$) in a* values. A two-way interaction was found between day and antioxidant ($P = 0.0003$) (Fig. 3). The antioxidant group consistently had higher redness throughout the trial, with each day decreasing in value; however, the day 3 control values were the same for day 4 values for the patties with antioxidants, implying that with the antioxidant treatment, a day of retail display may be gained in terms of redness.

![Fig. 1. Two-way interaction of antioxidant/no antioxidant treatment groups and display day on malondialdehyde concentration ($P < 0.0001$). Least square means without a common letter differ ($P < 0.05$).](image-url)
There was also no interaction between day and light ($P > 0.05$) or between light and antioxidant ($P > 0.05$) in $b^*$ values. A two-way interaction was found between day and antioxidant ($P = 0.008$) (Fig. 4). On day 3, the control treatments had similar $b^*$ to the antioxidant treatments on day 4. This trend can be seen throughout the rest of the study, with the antioxidant group reaching similar yellowness one day behind the treatment group. A main effect of lighting temperature was also expressed ($P = 0.0234$), stating that lower lighting intensity resulted in higher yellowness regardless of day or antioxidant.

There was no interaction between day and light ($P > 0.05$) or between antioxidant and light; however, a hue angle day and antioxidant interaction was present ($P = 0.0008$) (Fig. 5). On day 3, control patties had similar values as antioxidant patties on day 4 and continued in a similar pattern until the end of the trial. This once again presents the notion that with the introduction of antioxidants, there could

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**Fig. 2.** Two-way interaction of antioxidant/no antioxidant treatment groups and 3000K/3500K lighting intensity on $L^*$ value ($P = 0.0029$). Least square means without a common letter differ ($P < 0.05$). $L^*$ is a measurement of lightness on a scale of 0–100 with white representing 100.

**Fig. 3.** Two-way interaction of antioxidant/no antioxidant treatment groups and display day on $a^*$ value ($P = 0.0003$). Least square means without a common letter differ ($P < 0.05$). $a^*$ is a measurement of redness with positive values indicating red and negative values indicating green.
be an increase of one day in the display life of the product in regard to the prevention of instrumental discoloration.

There was no main effect of light found ($P > 0.05$) regarding chroma. There was no interaction between day and light ($P > 0.05$) or between antioxidant and light ($P > 0.05$), but there was an interaction between antioxidant and day ($P = 0.0008$) (Fig. 6). At day 3 in the control patties, the saturation values were similar to day 4 values in the antioxidant group. As seen with the other measurements, using the essential oil prolonged the degradation of the chroma values, which could provide an additional day during retail display in regard to the saturation of meat color. There was no light main effect presented ($P > 0.05$).

**Conclusions**

Throughout the duration of this study, antioxidants continued to behave in similar ways as recorded in the

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**Fig. 4.** Two-way interaction of antioxidant/no antioxidant treatment groups and display day on $b^*$ value ($P = 0.008$). Least square means without a common letter differ ($P < 0.05$). $b^*$ is a measurement of yellowness with positive values indicating yellow and negative values indicating blue.

**Fig. 5.** Two-way interaction of antioxidant/no antioxidant treatment groups and display day on hue angle ($P < 0.0001$). Least square means without a common letter differ ($P < 0.05$). Hue angle is a relationship between $a^*$ and $b^*$ values, with smaller angles representing a redder color and larger angles representing a more yellow color.
literature, regardless of the introduction of lighting intensities. The introduction of antioxidants allowed for the prolongation of color values throughout a display period; however, based on the results of this study, there was no relationship between antioxidant use, lighting intensity, and day. When considering different means of maintaining meat color, antioxidants can be utilized to achieve this goal. The antioxidant effect found in essential oils such as rosemary continues to prove similar results as predicted regardless of lighting intensity. Adding an antioxidant to ground beef decreases lipid oxidation over a period of five days as compared to ground beef that has not been treated. Antioxidants reduce lipid oxidation regardless of the lighting intensity. As retailers and consumers continue to search for more ways to reduce the oxidation of their meat products and increase the shelf life, antioxidants can be used in ground products to achieve these results.

**Acknowledgments**

This research project was funded by the Bumpers College Undergraduate Research and Creative Project Grant.

**Literature Cited**


![Fig. 6. Two-way interaction of antioxidant/no antioxidant treatment groups and display day on chroma value (P = 0.0008). Least square means without a common letter differ (P < 0.05).](image-url)
I was born and raised in Fayetteville, Arkansas. I graduated from Fayetteville High School in 2019. I am now graduating from the University of Arkansas with a major in Birth through Kindergarten with a minor in Agricultural Business. I came to the University of Arkansas knowing that I wanted to get accepted into occupational therapy graduate school. This is when I decided to go back to my roots and pursue my childhood dream of teaching as my undergraduate degree. I believe it was the best decision I could have made.

What drew me to the Birth through Kindergarten degree was the Jean Tyson Child Development Study Center (JT-CDSC). I was able to engage in hands-on learning throughout my college experience and be a part of an early childhood education classroom environment. This allowed me to practice interacting with and designing learning experiences for young children.

As I was nearing graduation, I wanted to find a way to give back to the community that helped shape me into who I am today. This was when I decided to implement an honors creative research project at the JTCDSC. In this way, I was able to build on my knowledge, give back to my community, and have a lasting impact on the infant and toddler students at the center.

I came up with this honors project when talking with my mentor, Dr. Laura Herold. What I did not expect was how big an impact it would have on me. Through lots of hard work, I learned a valuable lesson: when you do something you love, people will notice and success will follow.

Meet the Student-Author

Ellen Mathews

Fostering Infant and Toddler Music Competence at the Jean Tyson Child Development Study Center

Research at a Glance

- Music is a crucial part of supporting and extending infant and toddler development.
- Introducing musical instruments led to observations that suggest growth across many learning domains of development.
- The developmentally appropriate introduction of musical instruments in an outdoor learning environment fostered a positive learning community that will affect the growth and development of children for many years to come.
Fostering Infant and Toddler Music Competence at the Jean Tyson Child Development Study Center

Ellen Mathews,* Laura Herold,† Shelley McNally,§ and Donia Timby‡

Abstract

This paper presents a creative research project that introduced musical instruments in an outdoor setting to infants and toddlers ages 0-to-3 years old. It was grounded in research suggesting that music plays a vital component in expanding development in the early childhood years, helping to promote learning across many domains. This project began with a survey distributed to 7 infant and toddler classroom educators, after which responses were analyzed for themes regarding perceived infant interests. Subsequent observations were conducted to evaluate the best fit for the implementation of an outdoor experiential music space. Based on the findings, a developmentally appropriate musical space was implemented on the infant and toddler playground of the University of Arkansas Jean Tyson Child Development Study Center. Key elements of the space included a design allowing for open exploration, comfortable seating for all ages, and promoting conversations and interactions. Finally, observations were conducted to evaluate the impact of this setting on infant and toddler interactions. Qualitative analysis suggested that the new outdoor music space promoted interactions and engagement, dramatic play episodes, and the expression of emotions, which are all critical to the development of self-esteem and social-emotional competence.

* Ellen Mathews is a May 2023 honors program graduate with a degree in Birth through Kindergarten and a minor in Agricultural Business.
† Laura Herold, the faculty mentor, is an Associate Teaching Professor in the School of Human Environmental Sciences.
§ Shelley McNally is the Executive Director of the Jean Tyson Child Development Center and is an Associate Professor of Professional Practice.
‡ Donia Timby is a Senior Instructor of Human Development and Family Sciences in the School of Human Environmental Sciences.
**Introduction**

Research shows that music is a crucial part of child development (Adams and Parlakian, 2016; Baumgart and Kroll, 2018; Darrow, 2011; Luckenbill et al., 2019). Because music connects multiple domains of development, bringing music to an infant and toddler playground can help meet developmental needs across domains, promoting learning and development in the young learners with access to the space.

There are many advantages to incorporating music into students' early education experience, as it is a foundational art that can support all students' development. Music benefits emergent literacy and language development (Luckenbill et al., 2019), cognitive development (Adams and Parlakian, 2016), cultural awareness, and social and emotional learning (Baumgart and Kroll, 2018), and science and technology development (Baumgart and Kroll, 2018). Indeed, music can be incorporated across many learning domains (Darrow, 2011).

Music helps develop emergent literacy and language development skills through repetition and rhyming. Through exposure to music, students become familiar with vocal sounds, repetition, and rhyming (Playing with Music at Home, n.d.). It also promotes cognitive thinking through learning cause and effect. Being able to experiment and create conclusions based on experience is the foundation for building skills later in life (Luckenbill et al., 2019).

Playing music also promotes social and cultural awareness. When a student learns about their cultural history, they “develop a stronger sense of themselves, including their abilities to pursue their goals and tell their own stories” (Wright, 2019). In other words, music helps them to develop and build a sense of identity.

In addition, music supports emotional and social expression. Music can allow students to express the way they feel without using words. “[They] use instruments to portray symbolic representations of different feelings and situations. The ways that they design their sounds to represent specific patterns become a common language for all the students to connect with” (Baumgart and Kroll, 2018). This is important since expressing emotions is important for communicating and coping with emotions.

Research also indicates that music can impact learning in the domains of science and technology (Baumgart and Kroll, 2018). Correlations exist between students making music and their emerging concepts of science and technology. “They are like little engineers going around their environments...” (Baumgart and Kroll, 2018). Students can experiment with a variety of materials to form sounds that they desire. It shapes their experience of materials and the world around them.

**Materials and Methods**

Building on the abundance of evidence that music plays a vital role in promoting development for children ages 0-to-3 years old, data collection for this study took place at the University of Arkansas Jean Tyson Child Development Study Center (JTCDSC). Upon receiving institutional review board approval, a survey was distributed to educators in the infant and toddler classrooms at the JTCDSC. This survey was designed to reveal i) educator beliefs about the importance of music, ii) current practices related to exposure to music in infant and toddler classrooms, and iii) potential design ideas (layout, content, etc.) for an outdoor music area that would best meet the children’s needs and interests. There was a total of 6 questions in the survey. The first question asked about the ages of the children in the classroom. The subsequent open-ended questions sought to gather information on how the educators envisioned a functional and exciting outdoor music space.

A total of 7 classrooms were offered the survey, and 7 classrooms completed it. This meant that 100% of the students who use the playground were represented in this study. Surveying educators was the closest way to hear the voices of these very young children; as educators are closely connected with their students and routinely observe their behaviors, they are informed about children’s interests. Survey results influenced the purchasing and design of the playground to best fit children’s needs and interests. Specifically, educator responses revealed the high demand for drums to be purchased (requested in 86% of survey responses). Duplicates of instruments were provided so that multiple students could play with the same type of instrument at the same time.

Patterns of use of the space and levels of engagement were also gathered to evaluate the level of effectiveness and impact this research project had on the students, educators, and school community. This occurred through observations of the outdoor playground both before and after the musical exploration area’s introduction. Through the collection of objective and subjective running record observational notes, students’ patterns of use of the space, as well as their engagement levels, were tracked over three nonconsecutive days prior to and three consecutive days after the intervention. Pre-intervention observations helped track the level of engagement before the intervention and also informed the purchasing of developmentally appropriate project materials. Post-intervention objective observations allowed for comparisons of patterns of use of the space and levels of engagement. Refer to Fig. 1 to view the area before intervention.
Results and Discussion

Surveying the educators allowed for a space design that meets children’s developmental needs and interests as perceived by the educators, therefore allowing for the most effective use of the instruments purchased. With the help of funding ($2,750), opportunities for musical exploration were brought to life through a space for outdoor music experiences that were truly beneficial for all students to use. The purchased items were easily accessible for educators to identify and access. This was accomplished by having storage units and transparent plastic containers, with concise labeling and colored pictures. Refer to Fig. 2 to view the area after intervention. This project unified classrooms across the center and deepened student development. Incorporating music in an outdoor space on the JTCDSC playground allowed for multiple classrooms to interact together. Students were able to collaborate and create music together. The music could be heard by all who are outside, helping to create social connections and encourage communication.

Post-intervention objective observations allowed for comparisons of patterns of use of the space and levels of engagement. During qualitative analysis of the anecdotal records, particular attention was paid to the questions of whether students were engaging in the instruments, and if so, how and what were the perceived levels of engagement.

Content analysis of observational records indicated that students were engaging with the instruments. During observation, there was a substantial increase in the number of minutes spent in the music area. Student involvement in the music space grew from 36.7% pre-intervention to 84.4% post-intervention. Their level of observed engagement went from spending <1 min. in space pre-intervention, to >10 mins post-intervention (Table 1).
**Fig. 2.** Music space post-intervention at the University of Arkansas Jean Tyson Child Development Study Center.

**Table 1.** Observational data collected before (Pre) and after (Post) providing musical instruments in an area of the playground at the University of Arkansas Jean Tyson Child Development Study Center.

<table>
<thead>
<tr>
<th>Timing</th>
<th>Number of educators visiting music space</th>
<th>Number of students visiting music space</th>
<th>Avg. length of time students interacted with music space</th>
<th>Total number of students on playground</th>
<th>Total number of educators on playground</th>
<th>Length of time of observation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>1st observation</td>
<td>0</td>
<td>2</td>
<td>5</td>
<td>9</td>
<td>0.5</td>
<td>10</td>
</tr>
<tr>
<td>2nd observation</td>
<td>0</td>
<td>1</td>
<td>6</td>
<td>8</td>
<td>0.5</td>
<td>14</td>
</tr>
<tr>
<td>3rd observation</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Average</td>
<td>0</td>
<td>2</td>
<td>3</td>
<td>9</td>
<td>0.33</td>
<td>11.33</td>
</tr>
</tbody>
</table>
Engagement during the post-intervention observation included vignettes like the following that suggest that musical instruments aided in sense-making and communication in the largely preverbal children using the playground:

_A lawn mower began to run behind the fence. The environment became extremely noisy as the mower ran back and forth along the fence line. A few children on the playground began screaming and running. The children who had a drum began banging on the drum loudly and quickly. It began to feel like a chant, and the tone of the environment changed. After a few minutes, the banging slowed down, and the screaming and running quieted down. The mower moved further away. The moderate pace of drumming seemed to entice two students to run over to the space, and they began twirling and spinning right outside the back pergola. They waved their arms slowly up and down._

Students were using a variety of instruments. As drums were in highest demand and familiar to most students, they were the most utilized (Fig. 3). However, after some time spent in the music space, students began to explore other instruments like the bells, maracas, loofas, whisks, chimes, etc. (Fig. 4).

Results revealed students engaged in activities and conversations they were not able to prior to the intervention. Conversations between educators and students included how we treat new materials, how we keep ourselves safe, what the new unfamiliar instruments were, and how some instruments worked. By incorporating a variety of instruments with varying degrees of difficulty to manipulate, all students were intrigued and challenged, no matter their age and level of development.

In the future, this project will continue to impact the young students and educators at the JTCDSC. Opportunities also exist for expanding the outdoor music environment. Similarly, opportunities exist for expanding on the

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**Fig. 3.** Student utilizing instrument at the University of Arkansas Jean Tyson Child Development Study Center.
research presented here. Future studies should include longer-term observations, as opposed to the short-term observations featured in this study. Ideally, observations could be completed over several years as part of a longitudinal study to gather the full impact of fostering music in infants and toddlers. This would lead to a better understanding of how introducing instruments impacts all domains of development.

Conclusions

The goal of this project was to foster the growth of infants and toddlers by introducing musical instruments to a local community of young learners. The results of the project align with research indicating the proven positive effects that music has on the development of young infants and toddlers. Building on survey and pre-observation data, a developmentally appropriate outdoor music space was designed, incorporated, and then well-utilized by all students. Evidence from the post-observations clearly suggests increased engagement and opportunities for learning across domains among the infants and toddlers who used the space.

Acknowledgments

Funding was provided by the Student Undergraduate Research Fellowship (SURF) and Honors College matching.

Literature Cited


I graduated Summa Cum Laude in the spring of 2023 with a double major in Poultry Science and Environmental, Soil, and Water Science, and a minor in Soil Science. I am from Bentonville, Arkansas and graduated from Bentonville High School in 2019. I was a member of the Vietnamese Student Association and Crop, Soil, Environmental Science Club while also serving as an officer in both the Poultry Science Club and Bumpers Honors Student Board. These past two years, I worked at the Poultry Health Laboratory under Dr. Billy Hargis and in the Poultry Science Parasitology Lab under Dr. Danielle Graham, where I focused on *Histomonas meleagridis*, a parasitic protozoan in turkeys. I was named the 2023 USPOULTRY Frank Perdue Student of the Year. This fall, I will be attending Clemson University to pursue a M.S. degree in microbiology, focusing on the molecular aspects of *H. meleagridis*. I have always been fascinated by biology, which has played a large role in getting me to where I am today. I truly feel fortunate that I have been able to join the Bumpers family these past four years and have had a wonderful time along the way. Thank you to Dr. Gisela Erf for her excellent mentorship and guidance these past four years, especially throughout my Honors program, and to my committee, Dr. Billy Hargis and Dr. Guillermo Isaias Tellez, for their assistance in conducting my honors project and completing my honors thesis. Thank you to Aaron Forga and the Poultry Health Lab team for their assistance in bird rearing and care.

### Meet the Student-Author

Alessandro Rocchi

### Research at a Glance

- Heat stress is a growing concern within the poultry industry in both broiler production and animal welfare.
- Cyclic heat stress reduces circulating levels of T- and B-lymphocytes but not other blood cells in broiler chickens.
- Cyclic heat stress attenuates the acute local and systemic inflammatory response to lipopolysaccharide in broilers.
Effects of Cyclic Heat Stress on the Acute Inflammatory Response in Broilers

Alessandro J. Rocchi,* Chrysta N. Beck,† Jossie M. Santamaria,§ and Gisela F. Erf‡

Abstract

Heat stress (HS) is a growing concern in broiler production. Little is known regarding the effect of HS on immune function. To examine the effects of HS on innate immunity, the local- and systemic-inflammatory responses to lipopolysaccharide (LPS) were examined in Cobb 500 male broiler chicks reared under thermoneutral (TN) or cyclic HS conditions. Beginning at four days of age, HS birds were subjected to 35 °C from 8:00 a.m. to 10:00 p.m. and TN temperatures from 10:00 p.m. to 8:00 a.m. At 37 days of age, four groups of broilers were formed: LPS-TN (8 broilers), phosphate-buffered saline (PBS)-TN (4 broilers), LPS-HS (8 broilers), and PBS-HS (4 broilers), with each broiler receiving LPS- (100 µg/mL) or PBS-treatments by intradermal pulp-injection of 12 growing feathers (GF; 10 µL/GF). Blood and GF were collected before (0 h) and at 6 and 24 h post-injection to determine leukocyte population changes. Locally, LPS-HS broilers had lower (% pulp cells) levels of infiltrating heterophils and macrophages in GF-pulps at 6 and 24 h, respectively, compared to LPS-TN birds. In the blood, TN and HS broilers had similar baseline (0 h) concentrations of heterophils, monocytes, eosinophils, and basophils, but HS broilers had lower (P ≤ 0.05) T- and B-lymphocyte levels. Concentrations of heterophils and monocytes were greatly elevated (P ≤ 0.05) at 6 and 24 h, respectively, only in LPS-TN broilers. Overall, results indicated that cyclic HS reduced both the local and systemic acute inflammatory responses to LPS in broilers, likely impairing their innate defense against microbial infection.

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‡ Gisela F. Erf, the faculty mentor, is a professor in the Department of Poultry Science.
Introduction

There are two major parts to the immune system, consisting of innate and adaptive immunity. Innate immunity acts as the body’s first line of defense, developing ways for immediately dealing with pathogens through an inflammatory response. Inflammation works by accumulating “leukocytes, plasma proteins and fluid derived from blood” at the site of infection to eliminate microbes and repair damaged tissue (Abbas et al., 2018). To recognize infections, the innate immune system established a method for distinguishing molecular patterns common to groups of pathogens (e.g., lipopolysaccharide (LPS) of Gram-negative bacteria). With this, the innate immune system can quickly recognize pathogens and initiate inflammation (Medzhitov and Janeway, 1997).

The innate immune system is important for commercial broiler production as chickens are processed at six weeks of age before they can fully develop their adaptive immunities (French et al., 2020). The pulp of growing feathers (GF) has been shown to be an effective, minimally invasive skin test site to monitor local inflammatory responses in order to examine the effectiveness of the innate immune system (Erf and Ramachandran, 2016). Through simultaneous GF injection and periodic collection of injected GF, we may examine local leukocyte infiltration profiles and activities taking place in vivo. Changes in the blood may be determined from concurrently sampled peripheral blood.

Recently, a study using the “GF and blood dual-window approach” in broilers examined the acute inflammatory response to LPS injected into the GF pulp (French et al., 2020). This study showed extensive recruitment of heterophils and monocytes/macrophages in the dermis of injected GF, reaching peak levels at 6 and 24 h post-injection, respectively. Local GF cellular activities included the generation of reactive oxygen species (ROS), expression of inflammatory cytokines (e.g., interleukin-1 (IL-1), IL-6, IL-8, IL-10), and antioxidant enzyme activity (French et al., 2020). In the blood, concentration and proportions of heterophils were elevated at 6 h and returned to baseline levels by 24 h, whereas the proportions of lymphocytes dropped at 6 h and returned to pre-injection levels by 24 h (French et al., 2020). With the successful adaptation of this two-window approach for use in broilers, the influence of environmental conditions or nutrition on innate immune function in broilers may be investigated.

Heat stress (HS) is an environmental issue associated with broiler production, resulting in reduced feed intake and nutrient efficiency, along with increased water intake (Ruff et al., 2020). Little is known about the effects of heat stress on the innate immune system of broilers other than decreased gut barrier functions, allowing for bacterial translocation (Campbell et al., 2019). Examination of the local and systemic inflammatory response to LPS, similar to the study conducted by French et al. (2020), will provide a pertinent, novel understanding of the impacts of heat stress on the acute inflammatory response of broilers.

We hypothesize that birds subjected to cyclic heat stress will exhibit altered inflammatory responses when compared to broilers reared under thermoneutral conditions.

Materials and Methods

Newly hatched Cobb 500 broiler chicks were tagged at hatch and assigned randomly based upon their tag number to two different temperature treatment groups, thermoneutral or cyclic heat (TN or HS) (Gribbons and Herman, 1996). In total, eight environmental chambers were used, four TN and four HS. Each chamber was evenly split into two pens to produce eight pens per treatment (16 pens total). Twenty-three birds were placed into each pen on wood shavings with a stocking density of 10 birds/m². This study was conducted at the UA Poultry Environmental Research Laboratory (PERL). All protocols and procedures involving animals used in this trial were approved by the University of Arkansas System, Division of Agriculture, Institutional Animal Care and Use Committee (IACUC; protocol #21-018-2).

From Day 0 to 3, all birds were grown under the same temperature conditions of 32 °C. Cyclic HS conditions began on Day 4 for HS birds. HS birds were subjected to 35 °C from 8:00 a.m. to 10:00 p.m. (14 hours) and TN temperatures from 10:00 p.m. to 8:00 a.m. Temperature conditions for TN birds followed industry settings (i.e., Day 4–6, 31 °C; Day 7–10, 29 °C; Day 11–14, 26 °C; and Day 15 onwards, 24 °C). Diets followed industry standards, consisting of Starter from 0 to 10 days, Grower from 11 to 28 days, and Finisher from 28 to 42 days for all treatments. Lighting schedules followed industry standards for broilers with 24 h of light Days 0 to 1; 23 h of light with 1 h of dark Days 2 to 7; 20 h of light with 4 h of dark Days 8 to 14; and 18 h of light with 6 h of dark Days 15 to 42 for all treatments.

There were four treatment groups based on injection and temperature conditions: LPS-TN, phosphate-buffered saline (PBS)-TN, LPS-HS, and PBS-HS. Eight broilers per temperature group were used for LPS injection and four for PBS injection. Three broilers were selected randomly from each chamber, two for LPS (one per pen) and one for PBS (vehicle) injection.

When the broilers were 37 days of age, 6 GF from each breast tract were injected with 10 µL of LPS (100 µg/mL of PBS) or 10 µL of PBS (French et al., 2020). Six GF were collected before (0 h) and at 6 and 24 h post-GF pulp injection. Two GF were used to prepare pulp cell suspensions for direct immunofluorescent staining and cell population analysis by flow cytometry, as described by French et al. (2020).
Briefly, cell populations were identified using fluorescently labeled mouse monoclonal antibodies (mAb) for chicken leukocyte markers. Pulp cell suspensions were dual labeled for total leukocytes and macrophages using mAb CD45-SR and KUL01-FITC, respectively. A second dual labeling was used for B and T cell determination using Bu-1-FITC and CD3-PE, respectively (French et al., 2020). Heterophils were identified based on CD45-expression and granularity (side scatter characteristics) (Seliger et al., 2012). Data were expressed as percentages of leukocytes in the pulp cell suspension (% pulp cells).

At each time point, 1 mL of blood was collected from the wing vein using heparinized 3-mL syringes with 25-gauge x 1-inch needles (French et al., 2020). The blood was used for the preparation of Wright-stained blood smears to determine the proportions of lymphocytes, heterophils, monocytes, basophils, and eosinophils by microscopic evaluation of at least 300 white blood cells (WBC) per blood smear. Blood was also used to determine concentrations (cells/µL) of RBC, thrombocytes, WBC, heterophils, monocytes, and T- and B-cells in a whole blood assay. In this assay, various cell types were identified by direct immunofluorescent staining, and cell population analysis was conducted following a modified methodology of Seliger et al. (2012) on a BD C6-Plus flow cytometer (Becton Dickinson Biosciences, San Jose, Calif.). For both pulp and whole blood fluorescent staining, controls were included to detect non-specific binding of fluorescently labeled mAb, to determine cut-offs between positive and negative fluorescence, and to set compensations (French et al., 2020).

The concentration of eosinophils and basophils was calculated by multiplying the WBC concentration determined by flow cytometry by the percentage of eosinophils and basophils, as determined by manual differential leukocyte counting and dividing the product by 100.

Three-way analysis of variance (ANOVA) was conducted to determine the effect of treatment (PBS, LPS), temperature (TN, HS), and time (0, 6, 24 h) and their interactions. In the presence of significant interactions, data were separated into 10 groups, TN-0h, HS-0h, PBS-TN-6h, PBS-TN-24h, LPS-TN-6h, LPS-TN-24h, PBS-HS-6h, PBS-HS-24h, LPS-HS-6h, LPS-HS-24h and subjected to one-way ANOVA to detect group effects. Fisher’s least significant difference multiple means comparison analysis was conducted to determine differences between individual groups. Groups were considered different at \( P \leq 0.05 \).

**Results and Discussion**

Using the minimally invasive, “two-window approach” in broilers, French et al. (2020) were able to describe local and systemic inflammatory activities in response to intradermal (i.d.) pulp injection of LPS within the same individuals and over time. Similar to French et al. (2020), LPS administration in this study stimulated heterophil and monocyte/macrophage recruitment from the blood into the injected pulps. In TN and HS GF-pulps injected with LPS, heterophils reached peak levels (% pulp cells) at 6 h and remained above pre-injection levels at 24 h, whereas levels of macrophages were elevated at 6 h and continued to increase further by 24 h (Fig. 1). Baseline levels (0 h) of heterophils and macrophages in the pulp were similar in TN- and HS-broilers. However, pulp-infiltration was lower in LPS-HS compared to LPS-TN broilers at 6- and 24-h for heterophils and at 24 h for macrophages, indicating attenuated local inflammatory responses to LPS in broilers rear under cyclic HS conditions. Like in the French et al., 2020 study, lymphocytes were not recruited to the pulp by LPS injection and lymphocyte proportions tended to decrease (time main effect \( P = 0.003 \)) over the 24-hour post-injection period (Fig. 1). There were no changes in individual lymphocyte populations (i.e., T- and B-cells) following LPS injections. In both PBS-TN and -HS broilers, injection of PBS was not associated with significant changes in pulp heterophils or macrophages. For lymphocytes, pre-injection levels were greater than at 24 h only in PBS-TN broilers, although this drop was not significant for individual T- and B-lymphocyte populations (Fig. 1). Apart from the drop in lymphocytes for PBS-TN broilers, these results agree with observations reported by French et al. (2020). The small changes in leukocyte populations in PBS-injected GFs are likely due to inflammatory processes initiated by tissue damage associated with the injection and/or the stress of handling.

In LPS-TN broilers, the local inflammatory response was also reflected in blood cell profile changes (Fig. 1) that were similar to those reported by French et al. (2020). Specifically, concentrations of heterophils and monocytes were greatly elevated at 6 h. However, in LPS-HS broilers, there was no change in heterophil or monocyte concentrations in the blood over the 24-hour period (Fig. 1). The observations that LPS-HS broilers had lower GF-pulp infiltration of heterophils macrophages, together with a lack of elevation in blood heterophil and monocyte concentrations (cells/µL) post-injection, suggests an attenuated ability of LPS-HS broilers to meet demands of the inflammatory response (i.e., increased production and release of heterophils and monocytes from the bone marrow into the blood).

Interestingly, blood lymphocyte concentrations were greatly reduced at 6 h in both LPS-TN and LPS-HS broilers (Fig. 1). The drop in lymphocyte concentrations at 6 h in both groups of broilers was due to lower concentrations of T- and B-cells (Table 1). For both LPS-TN and LPS-HS broilers, T cell levels returned to baseline levels by 24 h, while B cell concentrations remained low. Moreover,
Fig. 1. Effects of cyclic heat stress (HS) on the proportions (% pulp cells) and blood concentrations \( (10^3/\mu L) \) of heterophils, macrophages, and lymphocytes in the pulp of growing feathers (GF) and blood at 0, 6, and 24 h post-intradermal injection of lipopolysaccharide (LPS). Pulps of 12 GF from each of 16 birds (8 thermoneutral (TN) and 8 HS) were intradermally (i.d.) injected with 10 µL of LPS (1 µg/GF; 12 GF/bird; 12 µg/bird) at 37-days of age. Pulps of 12 GF from each of 8 additional birds (4 TN and 4 HS) were i.d. injected with 10 µL of PBS. Blood (1 mL) and GF (2) were collected before and at 6 and 24 h post-GF injection. Pulp and blood cell suspensions prepared at each time point were used in a direct, dual labeling procedure using a panel of fluorescently labeled (FITC, PE, or SR) chicken leukocyte-specific mouse monoclonal antibodies, i.e., CD45-SR (total leukocytes/WBC), KUL01-FITC (monocytes/macrophages), Bu-1-FITC and CD3-PE (B- and T-cells, respectively). Cell population analysis was conducted using a Becton Dickinson C6-Plus flow cytometer. The lymphocyte population was calculated by adding B- and T-cell data. Heterophils were distinguished based on size (FSC) and granularity (SSC) characteristics of the leukocyte population (CD45+ cells). All data shown are means ± SEM. For each cell population, means without a common letter are different (P ≤ 0.05).
baseline concentrations (0 h) of T- and B-cells, and hence, lymphocytes, were lower in HS-broilers compared to TN-broilers (Fig. 1; Table 1). Hence, rearing broilers in cyclic HS conditions not only attenuated the expected increases in circulating levels of heterophils and monocytes in response to i.d. LPS injection but also reduced baseline levels of circulating lymphocytes (Table 1).

LPS and HS also affected the concentrations of other blood cells. This included a drop in RBC at 6 h in LPS-HS but not in LPS-TN broilers, and an increase in thrombocytes and a drop in eosinophils at 6 h in both LPS-TN and -HS broilers. There was no change in basophil concentrations in LPS-TN and LPS-HS broilers (Fig. 2). PBS injection had no effect on blood cell concentrations in either the PBS-TN or -HS group, except for elevated thrombocyte concentrations at 6 h and 24 h in PBS-HS and PBS-TN broilers, respectively. This change in thrombocyte concentrations may also be due to tissue injury caused by GF-pulp injection and/or handling stress. It should be noted that except for lymphocytes, the baseline levels of all other blood cells (i.e., RBC, thrombocytes, heterophils, monocytes, eosinophils, and basophils) were not different for broilers reared under TN versus HS conditions. Hence, it appears that cyclic HS alone did not affect hematopoiesis of myeloid cells. Rather, the lower concentrations of circulating T- and B-lymphocytes point towards an effect of cyclic HS on their development in the thymus and bursa of Fabricius, respectively.

In a similar study by Quinteiro-Filho et al. (2010), 35-day-old broilers were subjected to 10 h of HS (36 °C),...
with control birds kept at TN (21 °C) temperatures for 24 hours per day for one week. At 42-days of age, HS broilers were reported to have elevated serum corticosterone concentrations, decreased relative weights (% BW) of the thymus and bursa of Fabricius (primary lymphoid organs), and reduced Staphylococcus aureus-induced reactive oxygen species generation (ROS) by macrophages. These alterations in the thymic and bursal weights, as well as macrophage ROS generation, were attributed to the elevated levels of the stress hormone corticosterone (Quinteiro-Filho et al., 2010). Corticosterone, as well as sex steroids, are known to drive regression of primary lymphoid organs by reducing the levels of immature lymphocytes (e.g., CD4+CD8+ thymocytes) and hence, the weight of these organs. Moreover, corticosterone and other glucocorticoids are known to have anti-inflammatory properties, explaining the reduced ROS generation in response to S. aureus stimulation of macrophages in the Quinteiro-Filho et al. (2010) study.

While corticosterone concentrations were not measured in the current HS study, our observations of reduced local and systemic acute inflammatory responses and reduced circulating levels of T- and B-lymphocytes in HS broilers are likely due to elevated levels of HS-associated stress hormones. To gain a complete picture of the effects of cyclic HS stress on the LPS-induced inflammatory response, further studies are underway to examine functional activities of the cells recruited to the site of LPS injection, i.e., expression of cytokines and generation of ROS in GF-pulps, as well as changes in plasma proteins in response to i.d. LPS injection.

### Conclusions

Overall, the reduction in the LPS-stimulated, local and systemic acute inflammatory responses and in circulating concentrations of lymphocytes in HS broilers suggest that HS conditions impair the ability of broilers to mount effective innate immune responses. This may prove to be problematic for maintaining flock health as HS continues to grow as a major environmental concern. Further research should be done to elucidate the mechanisms and extent of this impaired immune function in HS conditions. Application of the “dual-window approach” could prove to be useful in selecting broilers exhibiting greater immune robustness while under the effects of HS.

### Acknowledgments

This work has been made possible by the Bumpers College Undergraduate Research and Creative Project Grant and the Honors College Research Grant (Rocchi). Additional funding was provided by the Tyson Endowed Professorship in Avian Immunology (Erf).

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**Table 1. Effects of intradermal injection with phosphate buffered saline (PBS) or lipopolysaccharide (LPS) on T- and B-cell concentrations in peripheral blood of broilers reared in thermoneutral or cyclic heat stress conditions.**

<table>
<thead>
<tr>
<th>Lymphocytes</th>
<th>PBS-TN</th>
<th>PBS-HS</th>
<th>LPS-TN</th>
<th>LPS-HS</th>
</tr>
</thead>
<tbody>
<tr>
<td>T cells (10^3 cell/µl)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 h</td>
<td>12.72 ± 0.58a,x</td>
<td>9.07 ± 0.59b,x</td>
<td>12.72 ± 0.58a,x</td>
<td>9.07 ± 0.59b,x</td>
</tr>
<tr>
<td>6 h</td>
<td>8.59 ± 0.98a,y</td>
<td>3.07 ± 0.81b,y</td>
<td>4.34 ± 0.78b,y</td>
<td>1.97 ± 0.28b,y</td>
</tr>
<tr>
<td>24 h</td>
<td>13.73 ± 0.99a,x</td>
<td>9.09 ± 1.16b,x</td>
<td>12.08 ± 0.70a,b,x</td>
<td>9.35 ± 0.70a,b,x</td>
</tr>
<tr>
<td>B cells (10^3 cell/µl)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 h</td>
<td>5.80 ± 0.26a,x</td>
<td>4.13 ± 0.32b,x</td>
<td>5.80 ± 0.26a,x</td>
<td>4.13 ± 0.32b,x</td>
</tr>
<tr>
<td>6 h</td>
<td>1.28 ± 0.31v</td>
<td>0.66 ± 0.18v</td>
<td>0.90 ± 0.13v</td>
<td>0.61 ± 0.06v</td>
</tr>
<tr>
<td>24 h</td>
<td>1.59 ± 0.29v</td>
<td>1.38 ± 0.25v</td>
<td>1.77 ± 0.22v</td>
<td>1.67 ± 0.31v</td>
</tr>
</tbody>
</table>

---

*One mL of heparinized blood was collected from the wing vein of 37-day old broilers before (0 h) and at 6 and 24 h post GF-pulp injection of PBS or LPS. Whole blood cell suspensions were prepared, and T- and B-cell populations were identified using fluorescently labeled mouse monoclonal antibodies CD3-PE and Bu-1-FITC to identify chicken T- and B-cells, respectively, in a dual direct labeling procedure. The proportions and concentrations of T- and B-cell populations were determined by flow cytometry.

† TN = Thermoneutral

‡ HS = Heat stress

Data shown are mean ± SEM; at 0 h, n = 12 broilers for TN/HS groups; at 6- and 24-h, n = 4 broilers for PBS-TN/-HS and 8 broilers for LPS-TN/-HS groups.

a, b: Lymphocyte concentration means within a row without a common letter are different (P ≤ 0.05).

x, y: For each type of lymphocyte, concentration means within a column without a common letter are different (P ≤ 0.05).
Literature Cited


An Inclusive Playground for Infant and Toddler Development

Meet the Student-Author

Amanda Swartz

I moved from The Woodlands, Texas, to attend the University of Arkansas in August 2019. I graduated high school from The Woodlands High. During high school, I worked at a pre-school daycare center which is where my initial passion for helping children started. I did not know exactly what career I wanted to embark on but I knew I wanted it to be centered around children and helping others, which ultimately led me to pursue my undergraduate degree in Human Development and Family Sciences. I joined a Greek life organization on campus, which later led me to attain 3 different officer positions. These positions were held from November 2020 to September 2022, during which time I got to lead over 200 women in various events and activities. I also completed over 250 service hours throughout my 4 years at the university, which included volunteer work with more than 5 different philanthropies in the Northwest Arkansas region. I received the Honors Research Grant when I began my service-learning creative honors project in August of 2022. I was awarded the Human Environmental Sciences Outstanding Student Award as well as named a Senior Scholar by Dale Bumpers College of Agriculture, Food and Life Sciences. This creative project could not have been done without the guidance of Dr. Jacquelyn Mosley and knowledge from Ms. Donia Timby. I thank both of them as well as Ms. Caitlyn Daniel and Dr. McNally from the Jean Tyson Child Development Study Center, for their assistance in the project.

Research at a Glance

• This project created more inclusive activities for children during outside playtime.

• The children received multiple benefits from music and art being added to their outdoor play experience.

• The stations stimulated growth in children’s cognitive development as well as other areas of growth.
An Inclusive Playground for Infant and Toddler Development

Amanda M. Swartz,* Jacquelyn D. Wiersma-Mosley,† Donia Timby,§
Shelley McNally,‡ and Caitlyn Daniel¶

Abstract

The purpose of this project was to help children reach more developmental goals and to make outdoor play at the Jean Tyson Child Development Study Center more inclusive for all children. Children gain many developmental goals from playing outside and being exposed to other environments as compared to just being inside the classroom. Outdoor play should be as inclusive as indoor play and offer many different activities and outlets, just as the indoor classroom does. The implementation of this service-learning creative project was to add more versatility to the outdoor area at the University of Arkansas Jean Tyson Child Development Study Center and to provide young children with more experiences outside the classroom in an outdoor space. The two outdoor stations that were developed focused on art and music and were under a roof beside the playground. These stations allow children other places to seek out when they do not want to or physically cannot run around on the other structures or want a more one-on-one social connection during outdoor playtime with teachers or peers.

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† Jacquelyn D. Wiersma-Mosley, the faculty mentor, is a Professor of Human Development and Family Sciences in the School of Human Environmental Sciences and Assistant Dean for Diversity, Equity, and Inclusion, Bumpers College.
§ Donia Timby is a Senior Instructor of Human Development and Family Sciences in the School of Human Environmental Sciences.
‡ Shelley McNally is an Associate Professor of Practice at the Jean Tyson Child Development Study Center, School of Human Environmental Sciences.
¶ Caitlyn N. Daniel is a Teaching Associate with the Jean Tyson Child Development Study Center, School of Human Environmental Sciences.
Introduction

Inclusion is becoming a more prominent topic in day-to-day conversations, especially in school, and how children are able to be included and inclusive with others (Mulholland and O’Connor, 2016). Inclusivity can have a lot of meanings, but in the context of this creative project, it focuses on all types of personalities and abilities of children in outdoor play (Moore and Lynch, 2015). Mental health benefits in outdoor play allow children to have better emotional stability because of their ability to run free, shout and blow off steam while being outside on the playground (Mahyok, 2015). To better understand the need for children’s development to be specifically stimulated in outdoor play, it is important to look at Jean Piaget’s theory of cognitive development. The theory discusses four stages that children enter, which can help explain how a child sees and understands the world around them through their cognitive development (Babak et al., 2019). The preoperational stage is all about starting to classify things as they appear through mental imagery and language, as well as symbolically thinking about words or objects (Meleod, 2020). These stages can give insight into how an educator can create an environment that promotes their students’ cognitive learning.

The University of Arkansas Jean Tyson Child Development Study Center (JTCDSC) is a learning center with infants ranging from 8 weeks old to 5 years old for the children of families in the Northwest Arkansas region, with students from the University of Arkansas attending as part of their coursework. This center was chosen because of its direct impact and influence on the University of Arkansas campus and because it strives to utilize child-first protocols for the best learning experiences. The purpose of the project was not only to create more inclusion but also to allow children to potentially meet more developmental standards. Early learning standards have been adopted by most states serving as guidelines for expectations and desires for children under the kindergarten level (National Association for the Education of Young Children, 2002). The additional areas outside will potentially give more experiences for those children to have an equal opportunity of achieving some of those standards during outdoor play as well.

Materials and Methods

In order to create stations that were unique but beneficial and developmentally appropriate, the basic school subjects were analyzed to see where the outdoor area was lacking. Art and music were the chosen subjects that were core to most curricula and also were, in reality, possible to bring outside, being durable through all forms of weather. Art and music also brought more creativity and imagination for outdoor use because they are abstract and offer limitless outlets for children.

Research on different music and art stations was conducted to investigate different structures and areas that could be created in the empty space given for this project. For the art station, an art easel with some shelves attached or a place to hold paint was ideal. An art easel was found (Community Playthings, Kansas City, Missouri) that had adjustable easel boards for accessibility for any child using it. The easel also had shelves for materials and a magnetic whiteboard backing for easy attachment of paper or utilizing the plain board for art. In the music station, there were not many options for a single structure that had all the components of instruments attached. The idea of putting a table with multiple music sets on top was a more practical idea unless a music structure, already built, was found at an affordable price. There were multiple music pieces that were found that had many different instruments attached. Three different music sets seemed to cover all bases to ensure there were a variety of components while also allowing multiple children to access the music station at one time.

An outdoor play area at the back of the Jean Tyson Child Development Study Center was identified. A portion of that area is covered by a roof extending over it. There is a porch with a ramp on one side and stairs on the other, all enclosed with a metal gate. The gate has openings to get from one side to the other and also to get down to the play areas. The covered porch will protect both the art and music stations from severe weather. The center desired more natural-looking wooden pieces because they hope to renovate the entire outdoor area in the future. It was also important that the director and educators at the Jean Tyson Child Development Study Center agreed and saw the need and benefit of implementing both stations. Both stations will require some adult supervision while being used, so the educators’ support in this project was necessary. Before bringing in the art and music stations, the current playground was photographed to have an idea of placement for the additions as well as to see what would fit and blend in the best with what the center has.

The art station was the first station introduced to the children during outside playtime. The reason behind this was the art easel, paint, and paper were expected to be easier for the children to be acclimated to, as there are not as many moving parts, and it is more self-explanatory and self-sufficient. The art station also gave teachers and caregivers time to adjust to the need for at least one adult to be supervising the station at all times while children were using different paints to ensure their appropriate use. The art easel was placed at the top of the porch, overlooking the playground. This placement was ideal because it was still guarded by the gate giving the art station its own space but also allowing children to overlook the playground for
**Fig. 1.** View of the area of the University of Arkansas Jean Tyson Child Development Study Center before the installation of the art station.

**Fig. 2.** View of the area of the University of Arkansas Jean Tyson Child Development Study Center after the installation of the art station.
potential inspiration for what they want to paint or draw. There were 11 different paint colors to choose from, as well as colored construction paper to use as a canvas for the art. The paper can be easily attached to the easel with the magnets provided, and there was a shelf at the bottom of the easel to rest paint, brushes, or sponges. There was also a bin added to sit underneath the easel to keep all the materials in one area so that no educator had to be responsible for carrying materials in and outside for playtime. (Figs. 1 and 2).

For the music area, there were three different sets, all composed of different instruments such as drums, xylophones, wooden noisemakers, tambourines, a wind chime, and bells. Each piece came with all the musical instruments detached and needed assembly. The instruments in the colorful and nature music set were all screwed in, so they were one piece. In contrast, the percussion set was composed in a way that all instruments are able to be removed and accessed individually. Having both options, with one set being permanently together and one having removable pieces, was ideal so that the children could either play by themselves or invite friends over to engage in the music together. All of the music pieces have legs that they stand on; however, in order for easier access, a table was needed to place the instruments on for better use. The area of the playground where the music station was set up was at the bottom of the porch. The location of the music station complemented the rest of the playground well because it was enclosed on both sides with gates as well as in the area that previously had sensory tables, so the children were familiar with it. Most of the musical instruments were detachable, so it was important for educators to continue to provide instructions and structure to the children on how to properly take care of this area of the playground so that the instruments do not wander outside of the designated area or get lost. (Figs. 3 and 4).

Results and Discussion

The purpose of this service-learning creative project was to develop spaces that bring about more options for children when they are outside for recess. There is research on the benefits children receive when connecting nature and art together in outdoor spaces. Specifically, outdoor art spaces seem to create more developmental opportunities as well as impact the health and well-being of children exposed to those environments (Moula et al., 2022). Art and music therapy have been used for years to aid children with behavioral, emotional, or other disorders and disabilities (Sze et al., 2004). Bringing music into daily educational settings has proven to help children in developing problem-solving skills, analyzing situations, and enhancing their creativity (Sze et al., 2004). As seen in the research, bringing art and music activities to the playground allows children to have more experiences and opportunities to achieve early learning standards (Moula et al., 2022). Outdoor play is pivotal for children’s developmental growth; the art and music stations will prove to assist with increasing developmental goals through the early learning standards. This project will help the learning standards in the areas of language, social, cognitive, and physical play (Arkansas Head Start, 2016). Students will be able to use gross and fine motor skills, interact with peers and educators, as well as learn responsibility through taking care of these stations and the objects that belong in them, which will fit into many components of the learning standards.

This creative project is only a small step in the right direction for the outdoor space at the center. Although there are two new spaces to provide more inclusion, in the future, the center should focus on remodeling the entire outdoor play structures to allow for more inclusion in the whole space. The limitations of this study were the need to use already-made products to add to the music station. The music station could have been improved if a structure could have been developed, made, and created solely from scratch to be able to fit every need, be durable, age-appropriate, and realistic with the children’s use of it. With the current set-up, the educators will have to be more attentive to ensure that the space is taken care of because of the many removable music pieces and the need to make sure that instruments stay in the area.

Conclusions

Finding how to create more advantages and benefits for children through scientific research regarding outdoor play and inclusion was the overall goal of this service-learning creative project. The Jean Tyson Child Development Study Center focuses on how to meet every child’s needs exactly where they are, and these additions to the outdoor playground helped continue to make that mission a reality. The children now have more access to activities that were once limited to just inside the classroom. Inclusive environments can encourage children to explore the world around them and ultimately allow them to prosper in their development.

Acknowledgments

This creative project was financially supported by an undergraduate honors research grant as well as physically supported by educators from the Jean Tyson Child Development Study Center.
Fig. 3. View of the area of the University of Arkansas Jean Tyson Child Development Study Center before the installation of the music station.

Fig. 4. View of the area of the University of Arkansas Jean Tyson Child Development Study Center after the installation of the music station.
Literature Cited


I am from Farmington, Arkansas. I will be graduating from the University of Arkansas in the Spring of 2023 with a degree in Horticulture and minors in Agriculture Communications and Agriculture Leadership. While at the University of Arkansas, I have learned practical skills and research in Horticulture. I have been working for the Fruit Breeding Program, under the direction of Dr. Margaret Worthington, since my sophomore year, during which I have had hands-on experience with research and networking opportunities. I attended the Southern Region American Society for Horticultural Science conference and competed in horticulture judging. This summer, I will attend the American Society for Horticultural Science conference and compete in an undergraduate oral research presentation competition. Senior year, I received the outstanding senior in Horticulture award and the Vail-Watts award. I served as a Bumpers College Ambassador for one year. I want to thank Dr. Margaret Worthington for her help on this project and her guidance throughout my collegiate career. I also want to thank Dr. John Clark, Carmen Johns, Lacy Nelson, Alexander Silva Cordoba, Dr. Jackie Lee, the fruit breeding team at the Fruit Research Substation, and the Horticulture Department for their support through countless hours of guidance. Lastly, I want to thank my husband and parents for always supporting my endeavors and being there to help with everything.

**Meet the Student-Author**

Isabella Vaughn

**Research at a Glance**

- Primocane-fruiting is a recessively inherited trait, which means developing a genetic marker could accelerate the breeding process.

- PF2 KASP is the first diagnostic molecular marker associated with a phenotypic trait (primocane-fruiting) that yields potential for economic importance in blackberries.

- A primocane-fruiting molecular marker would be effective for blackberry and raspberry breeders to effectively breed a crop that will work in various climates.
Validation of a Diagnostic Marker for Primocane-Fruiting in Blackberry

Isabella Vaughn,* Alexander Silva,† Carmen Johns,§
Lacy Nelson,‡ and Margaret Worthington¶

Abstract

Typical blackberries (Rubus subgenus Rubus) have perennial crowns and roots and biennial canes. The first-year canes (primocanes) are usually vegetative, while second-year canes (floricanes) produce fruit. Primocane-fruiting blackberries produce fruit on first-year canes and are desirable to growers because they potentially allow for a longer harvest season in temperate regions and enable production in tropical areas where no natural chill hours are accumulated. The development of molecular markers for desirable traits can potentially increase efficiency in blackberry breeding. However, to date, there are no diagnostic molecular markers for economically important traits in blackberries. Primocane-fruiting is recessively inherited, and tetraploid blackberries must have four copies of the primocane allele for the trait to be expressed. A single locus strongly associated with primocane fruiting was recently identified on chromosome Ra03, and a new KASP marker (PF2) was developed within this locus. In this study, we validated the performance of the new PF2 marker in a seedling population (Population 1937) segregating for primocane-fruiting at the University of Arkansas System Division of Agriculture's Fruit Breeding Program. In 2022, 170 seedlings in the population were evaluated for primocane-fruiting. Of these seedlings, 68 were floricane-fruiting, 86 were primocane-fruiting, and 16 could not be phenotyped due to poor plant growth. The PF2 marker correctly predicted the phenotype for 146 of 154 progeny that were scored in the 1937 population. Some of the inconsistencies between the marker prediction and the observed phenotypes could have been due to weak plants shaded out by neighbors or human error.

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§ Carmen Johns is an assistant fruit breeder in the Department of Horticulture.
‡ Lacy Nelson is a Program Associate in the Department of Horticulture.
¶ Margaret Worthington, the faculty mentor, is an Associate Professor in the Department of Horticulture.
Introduction

Blackberries (Rubus subgenus Rubus) and raspberries (Rubus idaeus) are distinctive among fruit crops because they have a perennial root system and crown and biennial canes. Typically, the first-year canes (primocanes) are vegetative, and the second-year canes (floricanes) bear fruit (Clark, 2008). Primocane-fruiting (PF) is a valuable trait that has the potential to extend the harvest season because primocanes typically flower later in the growing season than floricanes and do not need to accumulate chill hours prior to flowering. The University of Arkansas System Division of Agriculture's (UADA) Fruit Breeding Program released the ‘Prime-Jan®’ and ‘Prime-Jim®’, the first PF blackberry cultivars, in 2004 (Clark et al., 2005). Since then, many other PF cultivars have been released by the UADA Fruit Breeding Program and other public and private programs. Potential advantages of the PF trait include late-season fruiting period on primocanes from late summer to fall; potential to schedule production based on primocane management; potential of two crops on the same plant in the same year (floricane crop first, then primocane crop); reduced cost of pruning by mowing canes (primocane crop only); avoidance of winter injury; avoidance of rosette/double blossom disease; eliminated need for chilling hours (for primocane crop only); and production of blackberries in new geographic areas (Clark, 2008).

In blackberries, all PF cultivars are derived from the wild PF R. argutus accession ‘Hillquist’ (Clark and Finn, 2011). PF is recessively inherited, and all four copies of the PF allele must be present for the trait to be expressed in autotetraploid blackberries (Lopez-Medina et al., 2000). PF is an important commercial trait in blackberries and raspberries; however, a diagnostic marker has not been developed yet in either species. Developing a genetic marker for PF could accelerate the breeding process by allowing breeders to design crosses more effectively, cull seedling populations to keep only PF seedlings, and discard floricane-fruiting (FF) seedlings.

Genetic research in blackberries has been delayed relative to other economically important fruit crops because of challenges including polyploidy and multisomic inheritance (Foster et al., 2019). Fortunately, new tools for genetic research in polyploids (Bourke et al., 2018) and genomic resources for blackberries (Bruna et al., 2023) have recently enabled molecular research in this crop. A low-density microsatellite marker-based genetic map of a segregating breeding population created in 2013 suggested that the PF locus was located on linkage group 7 (Castro et al., 2013). However, a recent genome-wide association study conducted with UADA Fruit Breeding germplasm indicated that PF was controlled by a major-effect locus on chromosome Ra03. Based on these findings, the UADA Fruit Breeding team developed a Kompetitive Allele Specific PCR (KASP) marker for primocane-fruiting (PF2).

The goal of this research was to validate the performance of the PF2 marker in a biparental breeding population (Population 1937) segregating for PF. Specifically, our objectives were to (1) determine if the progeny of Population 1937 fit the expected 1:1 segregation ratio for PF and (2) determine if the PF2 marker is linked to the PF trait in this population.

Materials and Methods

The population chosen for this study (Population 1937) was derived from a cross between ‘A-2506T’, a floricane-fruiting blackberry selection with three primocane alleles, and ‘APF-409T’, a PF blackberry selection. One hundred seventy progeny from the 1937 blackberry seedling population were planted in the spring of 2020 at the University of Arkansas System Division of Agriculture's Fruit Research Station in Clarksville.

Each blackberry seedling in the 1937 population was planted at 45.72-cm spacing. To separate each individual plant, the selected primocanes from each plant were traced up from a crown and then given a tag number (1 through 170). This blackberry planting was untipped and was irrigated as needed using overhead sprinklers. The population was phenotyped for PF three times during the growing season, on 16 June 2022, 6 July 2022, and 1 August 2022. The population was phenotyped multiple times because the specific timing of primocane flowering varied from plant to plant due to various developmental and climatic factors that could trigger PF expression. PF was scored as a binary trait depending on the presence or absence of primocane flowers or fruit on the selected canes. Plants that were less than 1 m tall were scored as unknown phenotypes because the expression of the PF trait can be affected by weak plants or excessive shading by neighboring plants.

Young leaf tissue from primocane leaflets less than 1 cm long was collected from each parent and progeny primocane that was tagged on 16 June 2022, transported back to the Department of Horticulture, Fayetteville, in coolers, and stored in a freezer (-20 °C). DNA was extracted following a modified CTAB protocol (Porubski et al., 1997). Genomic DNA from the 1937 population seedlings and parents was then sent to LGC (LGC Genomics, Beverly, Mass., USA) to be genotyped with the PF2 marker. KASP marker reactions were performed as described by Varanasi et al. (2022). Fluorescence signals provided from each sample were converted to tetraploid dosage scores and used to create a cluster plot in the ggplot2 R package (Wickham, 2016).

Statistical analysis was performed with the Chi-squared test to determine if the progeny of population 1937 fit the 1:1 FF:PF segregation ratio expected for under normal random
Results and Discussion

Of the 170 progeny in population 1937, 86 were scored as PF, 68 as FF, and 16 as unknown. By performing a Chi-square test, we determined that the null hypothesis could not be rejected and the population fit the expected segregation ratios for both random chromosome assortment (1:1 expected, $\chi^2 = 2.104$, $P = 0.147$) and random chromatid assortment (13:15 expected, $\chi^2 = 0.320$, $P = 0.571$) scenarios (Table 1). Similar results were obtained by Lopez-Medina et al. (2000), who found that many of the 36 populations studied fit expected segregation ratios for both random chromosome assortment and random chromatid assortment and that random chromatid assortment was a better fit than random chromosome assortment for 17 out of 36 populations.

When HEX and FAM fluorescence data were converted to tetraploid dosage scores, 6 progeny were scored as CCTT, 75 progeny as CTTT, and 89 progeny as TTTT (Table 2, Fig. 1). The female parent (A-2506T) was CTTT and the male parent (APF-409T) was TTTT. Thus, the T allele was determined to be the recessive PF allele, and the C allele was the dominant FF allele. For standard random chromosome segregation, the expected ratio of genotypes would be 0 CCTT: 85 CTTT: 85 TTTT, while the expected segregation ratio for random chromatid assortment would be 6.07 CCTT (1/28): 72.85 CTTT (12/28): 91.07 TTTT (15/28). It was impossible to conduct the Chi-squared test for random chromosome assortment with the PF2 marker data because one of our three genotype classes had no expected progeny under this scenario. However, our data fit the expected segregation ratio for random chromatid assortment almost perfectly ($\chi^2 = 0.111$, $P = 0.946$) (Table 2). The presence of progeny with two C alleles (CCTT) can only be explained by possible pollen contamination, accidental self-pollination of the female parent, or double reduction. Double reduction is the transmission of sister chromatids on the same gamete. It is only observed in polyploid species under random chromatid segregation and for loci that are unlinked to the centromere (Allard, 1960). Previous phenotypic data on 36 biparental populations suggested that double reduction may occur in tetraploid blackberry (Lopez-Medina et al., 2000). Furthermore, evidence for random chromatid assortment and double reduction has also been found in autoploid potato (Bourke et al., 2015) and yellow cress ($Rorippa$ spp.) (Stift et al., 2008) using molecular marker data. It should be possible to confirm whether these six CCTT seedlings are actually true seedlings of the A-2506T x APF-409T cross by genotyping with more molecular markers in the future.

The PF2 marker incorrectly predicted the phenotype for only 8 of the 154 progeny that were assigned a score as PF or FF in 2022 (Fig. 1). Five progeny that had a CTTT genotype were scored as PF, and three progeny with a TTTT genotype were scored as FF. It is possible that the

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**Table 1.** The 1937 population was scored as primocane-fruiting (PF), floricane-fruiting (FF), or unknown for the 170 progenies. The Chi-square ($\chi^2$) test was performed to determine the population fit the expected segregation ratios for both random chromosome assortment and random chromatid assortment.

<table>
<thead>
<tr>
<th>Population</th>
<th>Parental genotypes</th>
<th>No. FF progenies</th>
<th>No. PF progenies</th>
<th>Expected ratio</th>
<th>$\chi^2$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-2506 x APF-409T</td>
<td>Aaaa:aaaa</td>
<td>68</td>
<td>86</td>
<td>1:1</td>
<td>2.104</td>
<td>0.147</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Population</th>
<th>Random chromosome assortment</th>
<th>Random chromatid assortment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Expected ratio</td>
<td>$\chi^2$</td>
</tr>
<tr>
<td>A-2506 x APF-409T</td>
<td>13:15</td>
<td>0.320</td>
</tr>
</tbody>
</table>

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**Table 2.** HEX and FAM fluorescence data were converted to tetraploid dosage scores. The data fit for expected segregation ratio for random chromatid assortment.

<table>
<thead>
<tr>
<th>Population</th>
<th>Parental genotypes</th>
<th>Number CCTT</th>
<th>Number CTTT</th>
<th>Number TTTT</th>
<th>Expected ratio</th>
<th>$\chi^2$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-2506 x APF-409T</td>
<td>CTTT:TTTT</td>
<td>6</td>
<td>75</td>
<td>89</td>
<td>1:12</td>
<td>0.111</td>
<td>0.946</td>
</tr>
</tbody>
</table>

$\chi^2 = \text{chi-square}$.
inconsistencies between the PF2 marker and phenotype data could be due to true recombination between the PF gene and the PF2 marker or mistakes in plant tissue collection or phenotype scoring because of the close plant spacing of the population. These 8 seedlings with inconsistencies between PF2 genotypes and phenotypes and the 16 seedlings that were not assigned a PF phenotype in 2022 because of small plant stature will be phenotyped and genotyped again in 2023 to validate 2022 data. The phenotypes that did not match the predictive marker will be rescored to validate 2022 data.

Conclusions

We identified a single locus strongly associated with PF on chromosome Ra03 and developed a new diagnostic KASP marker (PF2) based on a single nucleotide polymorphism (SNP) marker within the PF locus. This is the first diagnostic molecular marker associated with a phenotypic trait of economic importance to be developed in blackberries. The phenotypic and genotypic segregation ratios observed in the progeny of the 1937 validation population used in this study best fit expected ratios for random chromatin assortment. The double reduction landscape in blackberries should be further investigated by developing a high-resolution linkage map. The utility of the PF2 KASP marker for predicting PF phenotypes was demonstrated in this validation population. Further research should be conducted to investigate inconsistencies between the marker prediction and observed phenotypes and validate the performance of the PF2 marker in diverse breeding germplasm.

Fig. 1. The HEX and FAM fluorescence data for each sample genotyped with the PF2 marker was converted to tetraploid dosages, and the phenotypes were color coded. The marker incorrectly predicted the phenotype for only eight of the 154 progeny that were assigned a primocane-fruiting (PF) or florican-fruiting (FF) score.
Acknowledgments

This work was funded by the National Institute of Food and Agriculture, USDA Specialty Crop Research Initiative project “Tools for Genomics-Assisted Breeding of Polyploids: Development of a Community Resource” (2020-02585) and the National Institute of Food and Agriculture, USDA Agriculture and Food Research Initiative project “Genomic Breeding of Blackberry for Improved Firmness and Postharvest Quality” (2018-06274). Additional funding for this research came from Hatch Project ARK02599.

Literature Cited


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- There is no need to mimic the format of the finished journal. The Managing Editor will import your document into InDesign and format it in two columns and place tables and figures, etc.

- Report measurements in metric and other standard scientific units. Units or symbols that are likely to be unfamiliar to a general readership should be defined.

- The journal is web-only so COLOR figures and tables are encouraged. Each figure must be submitted as a color 300 DPI resolution JPG or PNG file at a standard figure width of at least 5 inches (select “constrain proportions” and height will default proportionally). The final size of figures will be adjusted by the editor to fit the page layout. Make sure that all text labels within the figure and x- and y-axis labels will be readable at the final publication size. Please use 11 point Calibri (a sans-serif font) for all figure text. Make sure all text used in figures and tables is in black not gray (which is the Microsoft default).

- Create tables using the Table function in Microsoft Word. Do not use tabs, spaces, and hard returns. This will result in the tables needing to be reformatted which allows the introduction of errors and could delay publication of your manuscript. Use a sans-serif 9 pt. font (e.g., Helvetica, Calibri) with title only in bold and centered above table; look at prior Discovery journals for capitalization style, table width, and horizontal (0.05 width) rule styles. Please do not put vertical ruling lines in the tables.

View helpful tips for creating tables at: https://aaes.uada.edu/files/2019/09/Table-guidelines.pdf

- Center figure captions below the figure in a 9 pt. sans-serif font such as Helvetica.

- Indicate footnotes for tables using sequential superscript lowercase letters (a, b, c, etc.). Place table footnotes below the last horizontal rule of the table. Footnotes used to clarify or annotate text should be placed at the bottom of the page in which the reference appears and indicated with sequential superscript numbers (1, 2, 3, etc.)

- Use a comma before the word and in a series: The U.S. flag is red, white, and blue.

**Parts of the Manuscript**

The title page should include the following:

- a concise, descriptive title
- authors’ first names, middle initials (if any), and last names (faculty sponsor should be listed as a coauthor)
- an abstract
- a footnote identifying each author by classification and major for students; rank and department for faculty and staff; and identify faculty sponsor or mentor.
Meet the Student-Author(s) and Project Highlights:
The Meet the Student-Author(s) section consists of a professional headshot (taken by Paden Johnson, padenj@uark.edu) of student author(s) as well as a short biography (240 words; 1400 characters with spaces) that tells readers about student author(s): (high school attended, activities and awards while at the university, etc.). Please see past issues for examples. This is the place to thank professors and advisors. For Project Highlights, we will need 3 brief bullet points (each 100 characters maximum, not including spaces) that clearly and succinctly explain the main takeaways of the research/project (i.e., overall what was done, significance, practical applications of findings) for a broad-based, non-technical audience. Please avoid using jargon and technical terms. We will need a photo of the student alongside these bullet points showing student-author(s) at work in the lab, field, traveling abroad, presenting a poster, receiving an award, etc. These photos will be loaded as supplemental files when submitting through the Discovery Journal location on ScholarWorks@UARK.

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

Introduction
The Introduction states the purpose of the study, the hypothesis, and pertinent background information in 500 words or less.

Materials and Methods
The Materials and Methods section describes the experimental design, materials used, statistical analysis (required), and any other details needed for another researcher to reproduce the study and to confirm the validity of findings and conclusions.

Results and Discussion
The Results and Discussion section presents appropriate data, but not all data, in text, tables, and figures and places the findings in context with other research in the field. The discussion emphasizes new and important aspects of the research and conclusions that follow from them. Include the implications and impact of the findings. Relate your findings to observations of other studies. State new hypotheses when warranted, but avoid unqualified statements not supported by your data.

Conclusions
The Conclusions section presents a brief (one paragraph) summation of the research project presented in the paper and the significance of the findings and practical applications. No references are necessary and please do not introduce new material not discussed previously in the paper.

Acknowledgments
The Acknowledgment section recognizes financial support (undergraduate research grants, etc.) and other assistance. Note support by any companies or parties with a vested interest in the research results. Please thank your advisor, other professors, co-authors, and other individuals who helped with your research in the Meet the Student-Author section NOT in Acknowledgments.

Literature Cited
The Literature Cited section lists the complete references corresponding to those cited in the text. Within the text, references are indicated by (Last Name, Year); e.g., (Jones, 2000) (Smith and Jones, 2000) (Brown et al., 2000; Finn, 1998). List the complete citation alphabetically (by the first author’s last name). Multiple citations of the same author are listed chronologically or by order of reference in the text if dated the same year.

It is required that references be written as follows: Author(s). Year. Title. Journal title. (month and date if appropriate); volume:pages. As below, no italics, (unless Latin phrase or word, which requires italics):

*Please note: for the first author, the initials come after the surname. For subsequent authors, the initials come before the surname.*

Book references are written as follows:

Authors or editors. Year. Title. Publisher, Place of publication. As below, no italics, (unless Latin phrase or word, which requires italics):

John Wiley and Sons, London.

Internet URL citations are written as follows:


**NOTE:** Please be very meticulous about the proper use of citations. All *Discovery* papers will be run through a check for plagiarism.

**Manuscript Submission**

Submit your Word manuscript (with page numbers and continuous line numbering) as an 8.5 × 11-in. document, with double-spaced, 12-pt. text, in a single column, to the *Discovery* journal on ScholarWorks@UARK by choosing the Submit Article option on the left side of the screen at:

https://scholarworks.uark.edu/discoverymag/

**DO NOT submit through the thesis part of ScholarWorks@UARK. You must submit from within the Discovery site.**

You will be prompted through instructions on what to upload. Please direct any questions to the Managing Editor, Gail Halleck, ghalleck@uark.edu, Division of Agriculture Communications.

If you do not have a professional quality (300 DPI resolution) headshot available, please email Paden Johnson at padenj@uark.edu to arrange an appointment to have your photo taken.

Unless otherwise indicated, the editor will correspond with the first author for revisions, approval of proofs, etc.

**NOTE:** The first author (student) must include a current and a forwarding e-mail address (or phone number) for contact outside the school year. Please complete the Student Contact Information that you will be prompted for when you submit through ScholarWorks@UARK. It will be loaded as a supplemental file.

https://aaes.uada.edu/student-summer-contact-form.pdf

**Supplemental Information Checklist**

- An abstract (you will copy and paste into a separate window but abstract must remain in your Word document as well).
- **Cover letter** stating your intent to submit (title of paper) to the *Discovery* journal with signatures of ALL co-authors included.
- **Summer contact form** (see above for website link).
- **Biographies** for each student author (see past issues for example of what to include) and Project Highlights bullet points.
• **Photos** (at least 300 DPI, if possible) of you performing your research in the field or lab; participating in internships; studying abroad; presenting at conferences, etc. for inclusion in our Meet the Student Author portion of each paper.

**Review Procedures**

Papers will be reviewed by a reviewer, and decisions registered as follows:

• Publish with minor revision
• Publish with acceptable major revision
• Reject

Written comments of reviewers will be provided to the author usually via track changes through Word. Student authors are expected to make revisions as part of the publication process. Students will be required to submit a separate file stating how each comment was addressed in the revision. If the student author disagrees with a suggestion, the rationale for not making a suggested change should be provided.

View an example of a response to reviewer document at:


When a paper is accepted “with revisions,” a revised manuscript will need to be submitted through ScholarWorks@UARK and the managing editor will approve a final draft for publication.
A special thank you to the faculty mentors, editorial board members, and graduate students that participated in this publication. Through teaching appointments in the Bumpers College of Agricultural, Food and Life Sciences, research appointments in the Arkansas Agricultural Experiment Station, extension appointments through the Cooperative Extension Service, and in collaboration with other University of Arkansas Fayetteville faculty, these individuals make Discovery a reality every year.

About the Dale Bumpers College of Agricultural, Food and Life Sciences
Bumpers College provides life-changing opportunities to position and prepare graduates who will be leaders in the businesses associated with foods, family, the environment, agriculture, sustainability and human quality of life; and who will be first-choice candidates of employers looking for leaders, innovators, policy-makers and entrepreneurs. The college is named for Dale Bumpers, former Arkansas governor and longtime U.S. senator who made the state prominent in national and international agriculture.

About the University of Arkansas System Division of Agriculture
The University of Arkansas System Division of Agriculture’s mission is to strengthen agriculture, communities, and families by connecting trusted research to the adoption of best practices. Through the Agricultural Experiment Station and the Cooperative Extension Service, the Division of Agriculture conducts research and extension work within the nation’s historic land grant education system. The Division of Agriculture is one of 20 entities within the University of Arkansas System. It has offices in all 75 counties in Arkansas and faculty on five system campuses.