

## **Effect of cyclic heat stress on the acute inflammatory response in broilers.**

### **Introduction**

Over time, the immune system has continued to develop and evolve due to the intense stress placed upon it by pathogens (Medzhitov & Janeway, 1997). There are two major parts to the immune system, consisting of the innate and adaptive immunity. Innate immunity acts as the body's first line of defense, whereas adaptive immunity is highly specific and protective. One of the innate immune responses developed for dealing with pathogens is the inflammatory response. Inflammation works by accumulating "leukocytes, plasma proteins and fluid derived from blood" to the site of infection to eliminate microbes and repair damaged tissue (Abbas et al., 2018). To recognize infections, the innate immune system has established a method for distinguishing molecular patterns common to groups of pathogens (e.g., lipopolysaccharide (LPS) of Gram-negative bacteria). In doing this, the innate immune system can quickly recognize a pathogen and initiate inflammation (Medzhitov & Janeway, 1997).

This innate immune system is important in the realm of commercial broiler production as chickens are processed at six weeks of ages before their own adaptive immunities have had time to develop (French et al., 2020). To examine the effectiveness of the innate immune system, the pulp of growing feathers (GF) was shown to be an effective minimally invasive skin test to monitor local inflammatory responses to bacterial cell structures, such as LPS (Erf & Ramachandran, 2016). Through the simultaneous injection of multiple GF on a bird, followed by periodic sampling of GF for laboratory analyses, we are able to examine leukocyte infiltration profiles and activities taking place *in vivo*. Because these leukocytes are recruited from the blood, changes in the blood cell profiles may be determined from concurrently sampled blood. Recently, one of the first studies using the "GF and blood dual-window approach" in broilers examined the acute inflammatory response to LPS injected into the pulp (French et al., 2020). For this study, blood and GF were sampled three times – before injection (0 h) and at 6 h and 24 h after LPS injection into GF pulps – to examine the acute phase of the inflammatory response to LPS (French et al., 2020). This study showed extensive recruitment of heterophils and monocytes/macrophages reaching peak levels at 6 and 24 h, respectively. Local cellular activities

included generation of reactive oxygen species (ROS), expression of inflammatory cytokines (e.g., interleukin-1 (IL-1), IL-6, IL-8, IL-10) and anti-oxidant enzyme activity (superoxide dismutase, SOD) (French et al., 2020). In blood, the concentration and proportions of heterophils were greatly elevated at 6 h and returned to baseline levels by 24 h, whereas levels 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 is a common environmental issue associated with broiler production, resulting in decreased feed intake and nutrient efficiency, as well as 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 heat stress will exhibit altered inflammatory responses when compared to broilers reared under thermoneutral conditions.

## **Objectives**

The following research objectives guided this study:

1. To establish whether there is a difference in blood- and GF-leukocyte profiles and activities in broilers reared under heat-stress compared to thermoneutral conditions.
2. To examine the local and systemic acute inflammatory response to LPS injected into the pulp of GF in broilers reared under heat-stress compared to thermoneutral conditions.

## **Materials and Methods**

*Experimental Animals and Rearing Conditions:* For this study, a quantitative design was used in the form of a true experimental methodology. True experimental methodology involves using multiple created groups with similar measured outcomes where the individuals of each group are randomly assigned and not manipulated in any way (Gribbons & Herman, 1996). Newly hatched Cobb 500 broiler chicks will be tagged at hatch and randomly assigned, based upon their tag number, to two different temperature treatment groups (Table 1), thermoneutral or cyclic heat (TN or HS) (Gribbons & Herman, 1996). In total, eight environmental chambers will be used, 4 TN and 4 HS. Each chamber will be evenly split into two pens to produce 8 pens per treatment (16 pens total). This study will be carried out at the UA Poultry Environmental Research Laboratory (PERL) with University of Arkansas Institutional Animal Care and Use Committee (IACUC) approval for all protocols and procedures involving animals used in this trial. The temperature conditions for the broilers during the 42 D growing period for TN and HS conditions will be as described in Table 1.

**Table 1.** Experiment set-up including temperature, diet and light schedules<sup>1</sup>.

<b>Days</b>	<b>TN Temperature, °C</b>	<b>HS Temperature, °C 8 am - 10 PM</b>	<b>HS Temperature, °C 10 pm - 8 am</b>
<b>1 to 3</b>	32	32	32
<b>4 to 6</b>	31	35	31
<b>7 to 10</b>	29	35	29
<b>11 to 14</b>	26	35	26
<b>15 to 42</b>	24	35	24

<sup>1</sup>Twenty three birds will be placed into each pen on wood shavings litter with a stocking density of 10 birds/m<sup>2</sup>. Temperature scheduling subject to change based upon welfare and happiness of broilers during trial early on. Temperature conditions based upon normal industry settings (TN) and industry settings experiencing environmental heat stress (HS).

Diet will consist of Rochell starter D 0-10, Rochell Grower D 11-28, and Rochell finisher D 28-42 for all treatments.

Lighting schedule of 24 h of light D 0-1; 23 h of light with 1 h of dark D 2-7; 20 h of light with 4 h of dark D 8-14; and 18 h of light with 6 h of dark D 15-42 for all treatments.

*Experimental Induction of the Inflammatory Response:* There will be four treatment groups each consisting of 8 broilers based on injection and temperature conditions: LPS-TN, phosphate-buffered-saline (PBS)-TN, LPS-HS, and PBS-HS. Two broilers will be randomly selected from each pen with one for LPS and one for PBS (vehicle) injection. When the broilers are 37 days of age, 6 GF from each breast tract will be injected with 10  $\mu$ L of LPS (100  $\mu$ g/mL of PBS) or 10  $\mu$ L of PBS (French et al., 2020).

*Pulp Sample Collection and Processing:* GF (6) will be collected before (0 h) and at 6 and 24 h post GF pulp injection (French et al., 2020). Two of the GF will be used to prepare pulp cell suspensions for immunofluorescent staining and cell population analysis by flow cytometry (French et al., 2020). Cell populations will be identified using fluorescently labeled (FITC or PE) mouse monoclonal antibodies (mAb) for chicken leukocyte markers. Suspensions will be dual labeled for total leukocytes and macrophages using mAb CD45-PE and KUL01-FITC, respectively. A second dual labeling will be used for B and T cell determination using Bu-1-FITC and CD3-PE, respectively (French et al., 2020). Data will be expressed as percent of a leukocyte population in the pulp suspension. Using the pulp cell suspension, ROS generation shall be determined using a kinetic fluorescence assay using 2',7'-dichlorofluorescein-diacetate. (Rath et al., 1998). The remaining 4 GF will be placed in aluminum foil pouches and flash frozen in liquid nitrogen and stored at -80 °C for later cytokine analysis (IL-1, IL-6, IL-8 and IL-10) (French et al., 2020). The frozen GF will be equilibrated to room temperature to perform RNA isolation and synthesized into cDNA. cDNA will then be used in qPCR to analyze the relative expression of cytokine genes.

*Blood Sample Collection and Processing:* At each time point, 1 mL of blood will be collected from the wing vein using heparinized 3 mL syringes with 25-gauge x 1-inch needles (French et al., 2020). The blood will be used for 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 will also be used to isolate plasma to measure acute phase protein (Alpha-1-Acid Glycoprotein (AGP)) and inflammatory cytokine interleukin-1 $\beta$  by ELISA.

*Statistical Analyses:* Three-way repeated measures analysis of variance will be used to determine the effects of temperature (TN & HS), time (0, 6, & 24 h) and treatment (LPS & PBS) and their interactions followed by Tukey's multiple means comparison as appropriate. Statistical significance will be considered at  $P \leq 0.05$ .

### **Project Schedule**

Late August 2022 through Mid-September– Reserving space within the PERL to perform the experiment and gaining approval from IACUC.

Mid-September 2022 through October – Performing necessary feed calculations for feed to be ordered from University of Arkansas Division of Agriculture (UADA) feed mill. Completing order forms for birds required for research project and UADA-IACUC forms. Begin growing birds mid-October.

November 2022 – Continue with research project, growing birds until the end of November, including daily animal care (i.e., feeding, checking waterlines, and manual thermostat operation to ensure proper temperature conditions); injection and sampling on D 37, 38 and 39 and running time-sensitive analyses. End trial end of November.

December 2022 through Mid-February – Complete analysis of acquired flow cytometry and ROS data and continue data collection from ELISA, differential leukocyte counts, and qPCR. Perform statistical analyses of the data and compiling results to be evaluated.

Mid-February 2022 through April – Finish writing Thesis, submit research project for publication and defend Thesis.

## References

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- Ruff, J., Barros, T. L., Tellez Jr., G., Blankenship, J., Lester, H., Graham, B. D., Selby, C. A., Vuong, C. N., Dridi, S., Greene, E. S., Hernandez-Velasco, X., Hargis, B. M., & Tellez-Isaias, G. (2020). Research Note: Evaluation of a heat stress model to induce gastrointestinal leakage in broiler chickens. *Poultry Science* 99(3), 1687–1692. <https://doi.org/10.1016/j.psj.2019.10.075>
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### **Bumpers College Undergraduate Research Budget Form**

<b><u>Item</u></b>	<b><u>Cost</u></b>
<b>Monoclonal antibodies for immunofluorescence</b>	\$1000.00
<b>Reagents for ROS generation, buffers and enzymes, and general lab supplies</b>	\$1000.00
<b>AGP acute phase protein Assay</b>	\$500.00
<b>qPCR – Reagents and Instrument use cost</b>	\$1000.00
<b>Eliza reagents for interleukin- 1<math>\beta</math> analysis and instrument use cost for flow cytometry.</b>	\$1000.00

The main costs for this experiment are derived from the costs of different reagents and assays needed to properly analyze the blood and growing feather samples from the birds. The application for the Bumpers Honors Undergraduate Research Grant is planned to be used for the funding of “Eliza reagents for interleukin- 1 $\beta$  and instrument use cost for flow cytometry.” The other costs found on the budget form are planning to be accounted for by the Honors College Grant, which has also been applied for but has not granted funding yet.

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# ALESSANDRO ROCCHI

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<b>Education</b>	<b>University of Arkansas – Fayetteville, Arkansas</b> <ul style="list-style-type: none"><li>• <i>Expected to graduate Spring 2023</i></li><li>• Double Major: Poultry Science and Environmental, Soil, Water Science</li><li>• Minor: Chemistry</li><li>• Degree: Bachelor of Science in Agriculture</li><li>• GPA: 4.0</li><li>• Hours: 125</li><li>• Full Time Student</li></ul> <b>Honors Program</b> <ul style="list-style-type: none"><li>• Thesis topic: Alterations in local tissue and blood leukocyte profiles during the inflammatory response in heat stressed broilers.</li><li>• Technical Skill: Immunofluorescent staining of cell suspensions, flow cytometry, blood smear preparation &amp; Wright-staining, microscopy.</li></ul> <b>Educational Career Goals</b> <ul style="list-style-type: none"><li>• Complete my B.S.A at the University of Arkansas with a 4.0 GPA.</li><li>• Pursue a M.S. followed by a Ph.D. in Microbiology with the aim of becoming a researcher.</li></ul>
<b>Recognitions, Awards, and Scholarships</b>	<b>Chancellor's and Dean's List</b> <ul style="list-style-type: none"><li>• Fall 2019, Spring 2020, Fall 2020, Spring 2021, Fall 2021</li></ul> <b>Randal Tyson Endowed Scholarship</b> <ul style="list-style-type: none"><li>• 2020, 2021</li></ul> <b>Poultry Federation Scholarship Fund</b> <ul style="list-style-type: none"><li>• 2020, 2021</li></ul> <b>Fontaine R Earle Endowment</b> <ul style="list-style-type: none"><li>• 2021</li></ul> <b>C. Roy Adair Agronomy Scholarship</b> <ul style="list-style-type: none"><li>• 2021</li></ul> <b>Chancellor's Scholarship</b> <ul style="list-style-type: none"><li>• 2019 to Present</li></ul> <b>Governor's Distinguished Scholarship</b> <ul style="list-style-type: none"><li>• 2019 to Present</li></ul>
<b>Extracurricular Activities</b>	<b>Bumpers Honors Student Board</b> <ul style="list-style-type: none"><li>• 2021 to Present</li><li>• 2021 – Secretary</li><li>• Plan events to increase Student/Faculty interaction within the college.</li><li>• Serve as a voice for the student body to advocate for student needs within bumpers college.</li></ul> <b>Crop Soil Environmental Science Club</b> <ul style="list-style-type: none"><li>• 2021 to Present</li><li>• Attend monthly meetings with guest speakers and host events to increase public awareness of the environment.</li><li>• Participate in local stream clean ups.</li></ul> <b>Poultry Science Club</b> <ul style="list-style-type: none"><li>• 2019 to Present</li><li>• Attend monthly meetings with guest speakers, discuss different careers within the industry.</li></ul>

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	<b>Uark Vietnamese Student Association</b> <ul style="list-style-type: none"> <li>• 2019 to Present</li> <li>• Teach local communities about Vietnamese culture</li> <li>• Plan local events to promote Vietnamese pride and cultural awareness.</li> </ul> <b>International Culture Team</b> <ul style="list-style-type: none"> <li>• 2019 to Present</li> <li>• Present and learn about different culture to improve public speaking while spreading diversity and cultural intelligence.</li> </ul> <b>Fly Fishing Club</b> <ul style="list-style-type: none"> <li>• 2021 to Present</li> <li>• Attend monthly meetings to discuss fundraising opportunities and community outreach events.</li> <li>• Plan group trips to facilitate a community within the club.</li> </ul>
<b>Work/Volunteer Experience</b>	<b>Agriculture Lab Tech</b> <ul style="list-style-type: none"> <li>• 2021 to Present</li> <li>• Bird rearing.</li> <li>• Maintain lab duties to ensure biosecurity.</li> <li>• Biohazard waste management.</li> <li>• Assist in pathogenic lab trials.</li> <li>• Create lab growth media.</li> </ul> <b>Crystal Bridges Museum of American Arts</b> <ul style="list-style-type: none"> <li>• 2017 to Present</li> <li>• Volunteer</li> <li>• Grounds keeping, stockroom fulfillment, and Customer</li> </ul>
<b>Skills</b>	<ul style="list-style-type: none"> <li>• Fluent in Italian</li> <li>• General lab skills</li> <li>• Autoclave Operation</li> <li>• Photography</li> </ul>

## Unofficial Copy

Name: **Alessandro Rocchi**  
Student ID: **010878809**

Institution Info: The University of Arkansas  
Print Date: 2022-03-15

### Beginning of Undergraduate Record

**Fa 2019**

Bumpers Col Agri,Food,Life Sc  
Poultry Science Major  
Honors Program Honors

Term Honor: Chancellor's and Dean's List

Course	Description	Attempted	Grade	Points
AFLS 1023H	BUMPERS HNRS PERSPECTIVES	3.00	A	12.000
ANSC 1032H	HON INTRO ANIMAL SCIENCES	2.00	A	8.000
CHEM 1101L	UNIVERSITY CHEMISTRY I LAB	1.00	A	4.000
CHEM 1103	UNIVERSITY CHEM I	3.00	A	12.000
ENGL 2003	ADVANCED COMPOSITION	3.00	A	12.000
POSC 410V	SPECIAL TOPICS POULTRY SC POULTRY SCIENCE CAREERS	4.00	A	16.000

Test Credits Applied Toward Bumpers Col Agri,Food,Life Sc

Course	Description	Attempted	Grade	Points
BIOL 1543	AP: PRINCIPLES OF BIOLOGY	3.00	CR	0.000
BIOL 1541L	AP: PRIN OF BIOLOGY LAB	1.00	CR	0.000
ECON 2023	AP: PRIN OF MICROECON	3.00	CR	0.000
ECON 2013	AP: PRIN OF MACROECON	3.00	CR	0.000
ENGL 1013	AP: COMPOSITION I	3.00	CR	0.000
ENGL 1020	ENGL 1023 EXEMPTION	0.00	--	0.000
ENGL 1213	AP: INTRO TO LITERATURE	3.00	CR	0.000
HIST 2003	AP: HIST AMER PEOPLE TO 1877	3.00	CR	0.000
HIST 1113	AP: INST & IDEAS WORLD CIV I	3.00	CR	0.000
MATH 2554	AP: CALCULUS I	4.00	CR	0.000
PHYS 2054	AP: UNIVERSITY PHYSICS I	4.00	CR	0.000
PLSC 2003	AP: AMER NATIONAL GOVT	3.00	CR	0.000
PSYC 2003	AP: GENERAL PSYCHOLOGY	3.00	CR	0.000

Term GPA	4.000	Term Totals	Attempted	Earned	Points
			16.00	16.00	64.000
Cum GPA	4.000	Cum Totals	16.00	52.00	64.000

Good Standing

**Sp 2020**

Bumpers Col Agri,Food,Life Sc  
Poultry Science Major  
Honors Program Honors

Term Honor: Chancellor's and Dean's List

Course	Description	Attempted	Grade	Points
AGED 3143	COMMUNICATING AG TO PUBLIC	3.00	A	12.000
CHEM 1121M	HNRS UNIVERSITY CHEM II LAB	1.00	A	4.000
CHEM 1123H	HNRS UNIVERSITY CHEM II	3.00	A	12.000
COMM 1003	BASIC COURSE: FILM LECTURE	3.00	A	12.000
ENSC 1001L	ENVIRONMENTAL SCIENCE LAB	1.00	A	4.000
ENSC 1003	ENVIRONMENTAL SCIENCE	3.00	A	12.000
POSC 3513H	HNRS CURRENT APPROACH AGRI LAB	3.00	A	12.000

Term GPA	4.000	Term Totals	Attempted	Earned	Points
			17.00	17.00	68.000
Cum GPA	4.000	Cum Totals	33.00	69.00	132.000

Good Standing

**Fa 2020**

Bumpers Col Agri,Food,Life Sc  
Poultry Science Major  
Environmental, Soil and Water Science Major  
Honors Program Honors

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Name: **Alessandro Rocchi**  
Student ID: **010878809**

Term Honor: Chancellor's and Dean's List

Course	Description	Attempted	Grade	Points
AFLS 3413H	HNRS PROPOSAL DEV RES METH	3.00	A	12.000
BIOL 2011L	GENERAL MICROBIOLOGY LAB	1.00	A	4.000
BIOL 2013	GENERAL MICROBIOLOGY	3.00	A	12.000
CHEM 3602M	HNRS ORGANIC CHEM I LAB	2.00	A	8.000
CHEM 3603H	HNRS ORGANIC CHEMISTRY I	3.00	A	12.000
POSC 2343	POULTRY PRODUCTION	3.00	A	12.000
STAT 2303	PRINCIPLES OF STATISTICS	3.00	A	12.000

Term GPA	4.000	Term Totals	Attempted	Earned	Points
			18.00	18.00	72.000
Cum GPA	4.000	Cum Totals	51.00	87.00	204.000

### Sp 2021

Bumpers Col Agri,Food,Life Sc  
Poultry Science Major  
Environmental, Soil and Water Science Major  
Honors Program Honors

Term Honor: Chancellor's and Dean's List

Course	Description	Attempted	Grade	Points
CHEM 3612M	HNRS ORGANIC CHEM II LAB	2.00	A	8.000
CHEM 3613H	HNRS ORGANIC CHEMISTRY II	3.00	A	12.000
CSES 2201L	SOIL SCIENCE LABORATORY	1.00	A	4.000
CSES 2203	SOIL SCIENCE	3.00	A	12.000
ENSC 3003	INTRO TO WATER SCIENCE	3.00	A	12.000
POSC 2353	POULTRY BREEDER MGMT	3.00	A	12.000
POSC 4801	SEMINAR: RESEARCH TOPICS	1.00	A	4.000

Term GPA	4.000	Term Totals	Attempted	Earned	Points
			16.00	16.00	64.000
Cum GPA	4.000	Cum Totals	67.00	103.00	268.000

### Su 2021

Bumpers Col Agri,Food,Life Sc  
Poultry Science Major  
Environmental, Soil and Water Science Major  
Honors Program Honors

Course	Description	Attempted	Grade	Points
GEOS 1111L	PHYSICAL GEOLOGY LAB	1.00	A	4.000
GEOS 1113	PHYSICAL GEOLOGY	3.00	A	12.000

Term GPA	4.000	Term Totals	Attempted	Earned	Points
			4.00	4.00	16.000
Cum GPA	4.000	Cum Totals	71.00	107.00	284.000

Good Standing

### Fa 2021

Bumpers Col Agri,Food,Life Sc  
Poultry Science Major  
Environmental, Soil and Water Science Major  
Honors Program Honors

Term Honor: Chancellor's and Dean's List

## Unofficial Copy

Name: **Alessandro Rocchi**  
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Course	Description	Attempted	Grade	Points
CSES 1203	INTRO TO PLANT SCIENCES	3.00	A	12.000
CSES 4224	SOIL FERTILITY	4.00	A	16.000
ENSC 4023	WATER QUALITY	3.00	A	12.000
POSC 3123	PRINCIPLES OF GENETICS	3.00	A	12.000
POSC 4314	EGG AND MEAT TECHNOLOGY	4.00	A	16.000
POSC 4811	SEMINAR: PROFESSIONALISM	1.00	A	4.000

### Undergraduate Career Totals

Cum GPA: 4.000 Cum Totals 89.00 125.00 356.000

End of Unofficial Copy

Term GPA	4.000	Term Totals	Attempted	Earned	Points
			18.00	18.00	72.000
Cum GPA	4.000	Cum Totals	89.00	125.00	356.000

Good Standing

**Sp 2022**

Bumpers Col Agri,Food,Life Sc  
Poultry Science Major  
Environmental, Soil and Water Science Major  
Honors Program Honors

Course	Description	Attempted	Grade	Points
ASTM 2903	AGHE APPL MICROCOMPUTERS	3.00		0.000
CHEM 3813	ELEMENTS OF BIOCHEMISTRY	3.00		0.000
ENSC 3933	ENVIRONMENTAL ETHICS	3.00		0.000
ENSC 4033	ANALYSIS OF ENV CONTAMINANTS	3.00		0.000
POSC 4343	POULTRY NUTRITION	3.00		0.000

Term GPA	0.000	Term Totals	Attempted	Earned	Points
			0.00	0.00	0.000
Cum GPA	4.000	Cum Totals	89.00	125.00	356.000

**GISELA F. ERF, PhD****Department:** Poultry Science**Institution:** Board of Trustees, University of Arkansas  
Division of Agriculture  
Fayetteville, AR 72701, USA  
Office phone: 479-575-8664  
Email: [gferf@uark.edu](mailto:gferf@uark.edu)**A. EDUCATION AND TRAINING**

Ph.D. 1988 Cornell University, Ithaca, New York, USA. Immunology  
M.S. 1983 University of Guelph, Guelph, Ontario, Canada. Reproductive Physiology  
B.S. 1981 University of Guelph, Guelph, Ontario, Canada. Animal Science  
Assoc. 1979 Nova Scotia Agricultural College, Truro Nova Scotia, Canada. Animal Science

**B. PROFESSIONAL EXPERIENCE**

2006-date Tyson Endowed Professor in Avian Immunology, UA, Fayetteville, AR  
2004-date Professor, University of Arkansas, Poultry Science, Fayetteville, AR  
1998-2004 Associate Professor, University of Arkansas, Poultry Science, Fayetteville, AR  
1994-1998 Assistant Professor, University of Arkansas, Poultry Science, Fayetteville, AR  
1988-1994 Assistant Professor, Smith College, Biological Sciences, Northampton, MA

**Related Honors and Awards**

2015 John W. White Outstanding Research Award, Division of Agriculture  
2012 Honors College Distinguished Faculty Award  
2010 Gold Medal Faculty Mentor, University of Arkansas  
2010, 13, 14 Outstanding Mentor, University of Arkansas  
2006-date First holder of the Tyson Endowed Professorship in Avian Immunology  
2006 UA Alumni Association Faculty Achievement Award for Research and Teaching  
2005 Jack Justus Excellence in Teaching Award, Bumpers College  
2005 Helene Cecil Leadership Award, National Award, Poultry Science Association  
2001-date Inducted member of the University of Arkansas Teaching Academy  
1999 Purina Mills Teaching Award, National Award, Poultry Science Association  
1999 John W. White Outstanding Teacher Award, Bumpers College

**C. RESEARCH/SCHOLARLY INTERESTS**

Primary research interest in understanding fundamental aspects of immune system development and function in poultry in the context of age, sex, nutrition, stress, genetics, and environmental factors. As part of this research effort, we developed a minimally invasive skin test using the growing feather as an in vivo test-tube. Using this test-system together with blood sampling, we are exploring local cellular/tissue responses to various test-materials (antigens, adjuvants, immunomodulators, nanomaterials, etc.) and relate the local response profiles to activities in the blood in the same individuals. Another major research interest is in understanding multifactorial, non-communicable diseases, such as autoimmune diseases, using the chicken model. Included are, the spontaneously occurring autoimmune vitiligo and uveitis in the Smyth line chicken, and, more recently, scleroderma/systemic sclerosis and Hashimoto's thyroiditis in the UCD-200/206 and OS chickens, respectively. Research projects with the avian autoimmune disease models focus on etiology and early immunopathological mechanisms. Furthermore, considering the multifactorial and truly spontaneous nature of disease onset and progression, efforts are underway to establish these autoimmune disease models for preclinical treatment testing.

**Publications - selected** \*Undergraduate Research/Honors student; #Graduate student with Erf Shi, F.#, and G. F. Erf. 2012. IFN-gamma, IL-21 and IL-10 co-expression in evolving autoimmune vitiligo lesions of Smyth line chickens. J. Invest. Dermatol. 132:642-649.

Erf GF, Ramachandran IR\*. 2016. The growing feather as a dermal test-site: comparison of leukocyte profiles during the response to *Mycobacterium butyricum* in growing feathers, wattles, and wing webs. Poult. Sci.: 95:2011-22. (editor's choice)

Erf GF, Falcon DM, Sullivan KA\*.,# , Bourdo SE. 2017. T lymphocytes dominate local leukocyte infiltration in response to intradermal injection of functionalized graphene-based nanomaterial. J. Applied Toxicol. 37:1317-24.

Sullivan KA\*.,# , Erf GF. 2017. CD4+ T cells dominate the leukocyte infiltrations response initiated by intra-dermal injection of phytohemagglutinin into growing feathers in chickens. Poult. Sci. 96:3574-80.

French CE\*.,# , Sales MA, Rochell SJ, Rodriguez A#, Erf GF. 2020. Local and systemic inflammatory responses to lipopolysaccharide in broilers: new insights using a two-window approach. Poult. Sci. 99:6593-6605.

Glenn, H\*, Mullenix GJ, Erf GF. 2021. Effects of a low crude protein diet with and without *Spirulina platensis* inclusion on white blood cell profiles in broilers. Discovery 21:38-44

Sorrick J\*, Huett W\*, Byrne KA\*.,# , Erf GF. Immunopathology, cytokine signature, and melanogenesis in choroids of vitiliginous Smyth chickens with and without vision impairments. Exp. Eye Res. (in revision).

#### **D. UNDERGRADUATE/ HONORS STUDENT RESEARCHERS & PROJECTS**

- last 5 years; (semester completed/expected completion)

Carmen Boessen. *Honors* (BIOL). "Time course investigation of leukocyte infiltration in response to intradermal injection of quantum dot nanoparticles in the growing feather" (Sp 2016).

Anne Celeste Gibson. *Honors* (BIOL). "*In vivo* monitoring of leukocyte infiltration in response to iron oxide nanoparticle injections into the dermis of growing feathers in chickens" (Sp 2016).

Chelsea Ellington. *Undergraduate Researcher* (POSC). "Effect of dietary micronutrient source and levels on the inflammatory response in broilers" (Sp, 2018).

Jossie Santamaria. *Undergraduate Researcher* (CSES). "Poultry Husbandry: daily care, handling, pedigree breeding, vaccinations, rearing, and selection of chicken research lines" (Sp 2019).

Guillermo Tellez. *Undergraduate Researcher* (POSC) "Immunology in the laboratory: using antibodies as a research tool" Sp 2020.

Heather Glenn. *Honors* (POSC) "Effects of a low crude protein diet with and without *Spirulina platensis* inclusion on white blood cell profiles in broilers" (Sp 2021).

Juan David Vargas - *Honors* (CHEM) "Local leukocyte infiltration in response to intradermal injection of liquid silicone in avian systems" (Sp 2021).

Ian Gilbert. *Honors* (ANSC). "Leukocyte infiltration following intradermal injection of lipoteichoic acid in chickens" (Sp 2022).

Katelyn Clark. *Honors* (POSC) "Temporal, phenotypic, and numerical characterization of thyroid infiltrating mononuclear cells during development of spontaneous autoimmune thyroiditis in Obese strain chickens" (Fa 2022)

Alessandro Rocchi. *Honors* (POSC) "Alterations in local tissue and blood leukocyte profiles during the inflammatory response in heat-stressed broilers" (Sp 2023).



March 14, 2022

Dear Members of the Bumpers College Undergraduate Research Grant Selection Committee:

It is a pleasure to write this letter in support of Alessandro Rocchi's Undergraduate Research Grant application. I have known Alessandro since the Fa 2019 as his Poultry Science (POSC) academic advisor and POSC Honors faculty representative. In Sp 2020, I was his teacher in "Current Approaches in Agricultural Laboratory Research" (POSC 3513H), and since Sp 2021, I am serving as his Honors mentor. I am very impressed by Alessandro's interest and commitment to education and learning. Alessandro put together a very challenging academic program pursuing the Bachelor of Science in Agriculture with a double-major in Poultry Science and Environmental, Soil and Water Science, a minor in Chemistry, along with the Bumpers College Honors program. Additionally, he is actively participating in a variety of high-impact extracurricular activities and is working as an undergraduate research assistant with Dr. Billy Hargis' research group. While Alessandro just started his third year at the University, he has already completed 125 credit hours with a 4.0 GPA. It is a joy to work with this talented student as mentor, advisor, and teacher.

Alessandro sought me out as Poultry Science advisor in part to help him design his Poultry Science program with special focus on science and research. He identified the POSC 3513 (H) as a first step towards this goal. In this undergraduate research course, Alessandro gained hands-on experience working with the chicken model on current cell and molecular laboratory approaches used by researchers across disciplines [cell isolation, identification, microscopy, immunofluorescent staining, cell population analysis by flow cytometry, immunohistochemistry, ELISA, DNA isolation, PCR, and production of recombinant proteins, etc.]. Additionally, he learned to keep a professional laboratory notebook and to report his findings in manuscript-style Result and Discussion sections. Alessandro was one of two first-year students in this course of mostly junior and senior Honors students. He excelled in all aspects of this course. He demonstrated excellent technical skills and conducted the various laboratory exercises efficiently, with great care and precision. I was particularly impressed by his comments and questions during class discussion and his written reports, which clearly reflected his outstanding ability to grasp, interpret, apply, and integrate new knowledge.

Through collaborative research with Dr. Hargis' Poultry Health group on heat-stress and its effects on tissue and blood leukocyte population profiles, I worked with Alessandro during sample collections. For this project, Alessandro's duties included managing the experimental set-up, daily care of experimental animals, and coordination of experiment days with various researchers. He did an excellent job for this Industry-funded project. At that time, Alessandro became interested in our research and initiated discussion on the proposed research project. He was particularly interested in applying the approach described in French et al. (2020), a first publication on broiler's local and systemic responses to intradermally injected LPS (a potent stimulator of the inflammatory response).

As described in his proposal, Alessandro seeks to explore how heat-stress affects the acute inflammatory response to LPS at the injection site and in the blood circulation in broilers. With the approaches outlined, he will fill a critical knowledge gap on the effects of heat-stress on innate immune system function. Through the POSC 3513(H) course, he has fundamental experience with the proposed methods, and, except for the cell-population and reactive-oxygen-species (ROS) generation assays, the laboratory procedures can be conducted at a later time with stored samples. Alessandro has hands-on experience with all aspects involving experimental animals and experiment set-up. On sampling days, my graduate students and I will help Alessandro with injections, blood and feather collections, same-day sample processing, and data acquisition by flow cytometry and kinetic fluorescence assay. Other procedures, like differential blood leukocyte counts, determination of tissue/plasma levels of inflammatory proteins by ELISA, cytokine expression by qPCR, will be carried out by Alessandro over the requested two semester duration of the project. As mentor, I will guide him through the procedures and data analyses, as well as writing a manuscript and Honor thesis. It is an ambitious project, but Alessandro is committed to carry out the project in its entirety, making the most of this opportunity. I have no doubt he will do an excellent job with all aspects and will complete the study on time. Through this project, Alessandro will make an important scientific contribution regarding the natural defenses of commercial broilers reared in heat-stress conditions. This new knowledge will find direct application in future studies on optimizing broiler health and well-being.

On a more personal note, Alessandro truly is a very bright young man. He has the intellect, maturity, resourcefulness, motivation, highest ethical standards, communication skills and commitment, to excel at all levels in his academic program. He has a very positive outlook on life, a nice sense of humor, and excellent interpersonal skills. Moreover, he clearly enjoys learning and enthusiastically seeks challenges and unique opportunities. I have no doubt that Alessandro will become a very successful scientist and researcher and make many positive contributions to science. I have no reservations in giving a student of Alessandro Rocchi's outstanding caliber my highest level of recommendation for a Bumpers College Undergraduate Research Grant award.

Sincerely yours,

A handwritten signature in black ink that reads "Gisela Erf". The signature is fluid and cursive, with the first name "Gisela" and the last name "Erf" clearly distinguishable.

Gisela Erf, PhD  
Tyson Endowed Professor in Avian Immunology





March 14, 2022

Bumpers Undergraduate Research Grant Award Committee

Dear Selection Committee Members:

It is my honor to write a letter of recommendation for the application of Alessandro Rocchi, Honors Undergraduate Student at the Department of Poultry science for the Undergraduate Research Grant Award. I have met Alessandro when he started working as an undergraduate student worker at the Poultry Health Laboratory in 2019. Since that earlier time, Alessandro displayed the mental ability to grasp and understand concepts that will serve him well in his future career as a scientist. In my 21 years, here at the University of Arkansas Alessandro has been one of the most outstanding undergraduates which I have known. Alessandro is easy to like and has always shown respect for his co-workers and superiors in the lab.

As a young undergraduate student, he has shown tremendous work ethic and is also always happy to lend a helping hand in other experiments and regularly volunteers to work weekends. Due to this, and his ability to quickly understand the goals of experiments, Alessandro was asked to assist in on coming experiments with several poultry companies in the field of intestinal inflammation and heat stress poultry models. His capacity to learn and dependability are traits that I greatly respect and honor. Students of his caliber are often difficult to find, and we are glad he is working with us.

Alessandro has shown a great interest in both research and heat stress. I personally believe that he will be a major contributor to academia during his career. Without reservation, I recommend him to the committee as an outstanding candidate for the Bumpers Undergraduate Research Grant Award.

If I can be of any further assistance, please do not hesitate in contact me.

Sincerely,

Guillermo Tellez, D.V.M., Ph.D.  
Research Professor

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