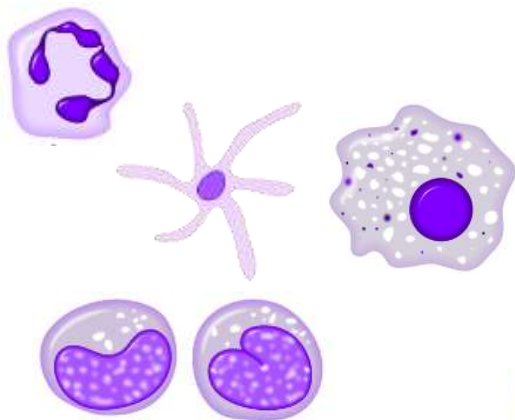


Host Defense Peptide-Medicated Disease Control and Prevention through Modulation of Innate Immunity



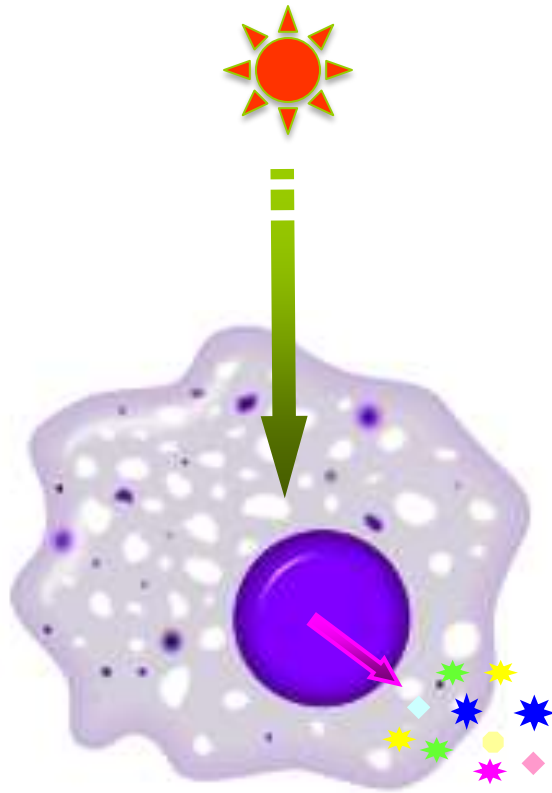
Guolong "Glenn" Zhang

Associate Professor

Department of Animal Science

OKLAHOMA STATE UNIVERSITY

Regulation of Immunity

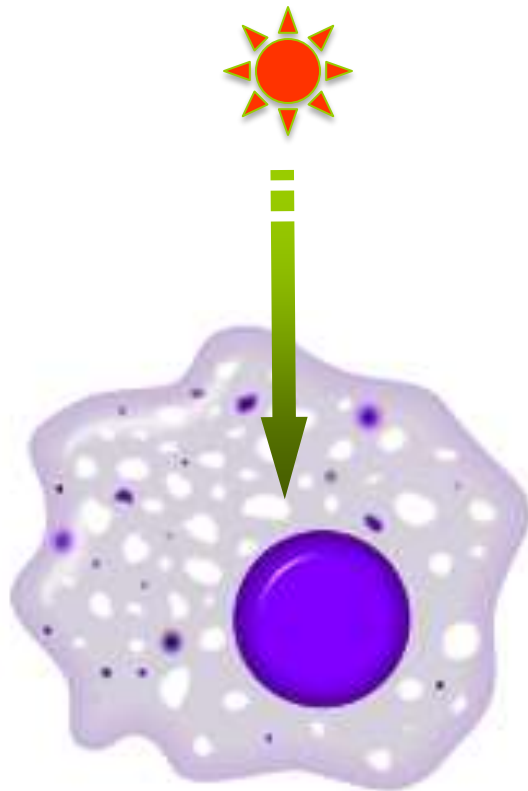


- Direct Delivery of effector molecules
- Indirect modulation of effector molecule production

Effector
Molecules

Anticicrobial Peptides/proteins
ROS and RNS
Cytokines/Chemokines
Costimulatory Molecules
Adhesion Molecules
Acute Phase Proteins

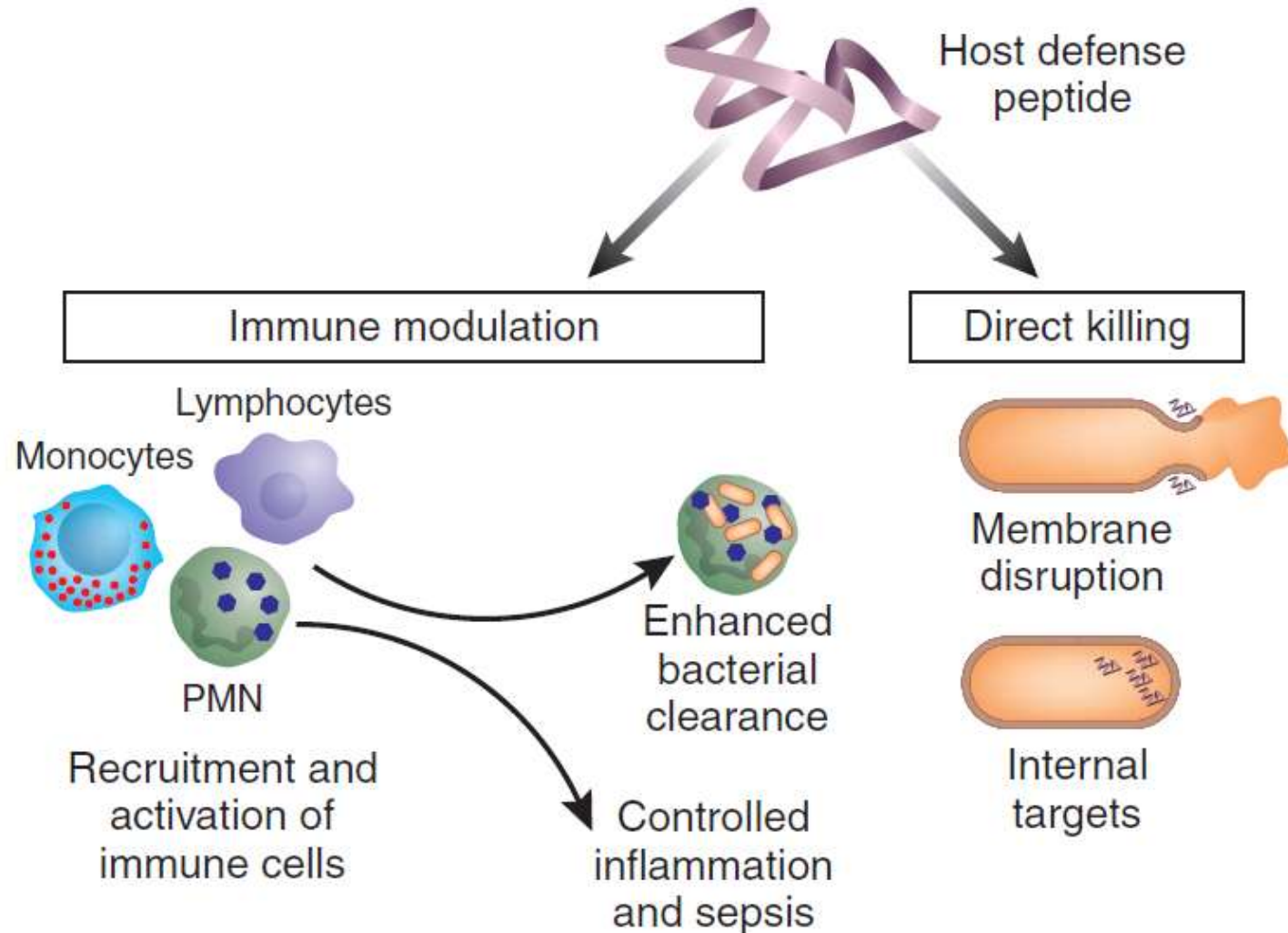
Immunoregulatory Strategies



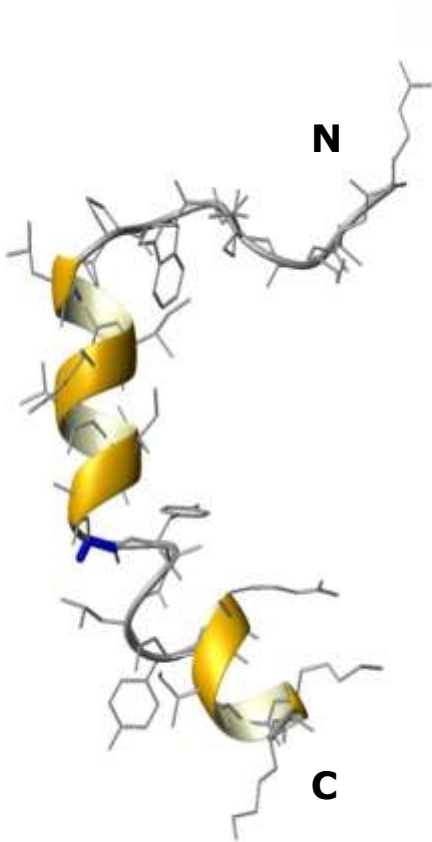
- **Direct Approach:** Delivery of novel HDPs to boost innate immunity and disease resistance
- **Indirect Approach:** Dietary modulation of HDP synthesis to boost innate immunity without triggering inflammatory response



Biological Functions of HDPs

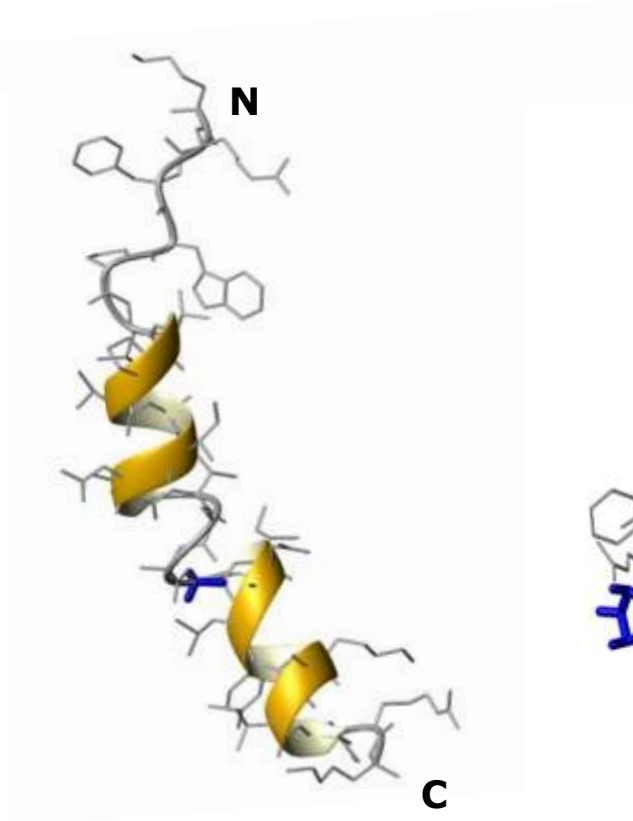


3-D Structure of Fowlicidins



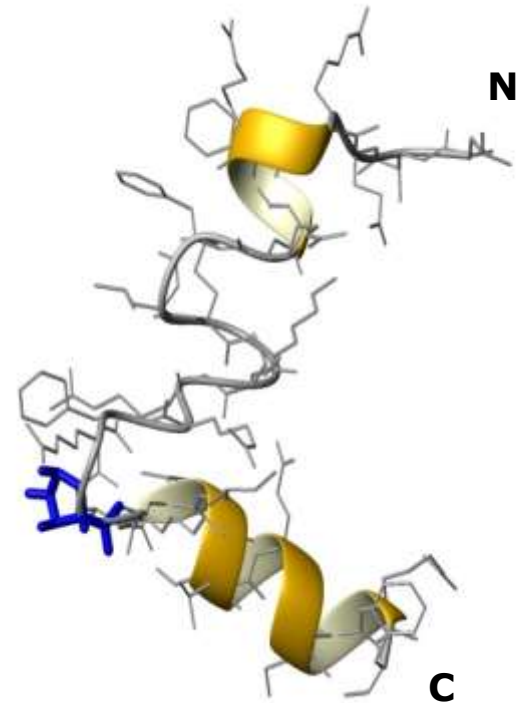
Fowlicidin-1

(FEBS J. 2006,
273: 2581)



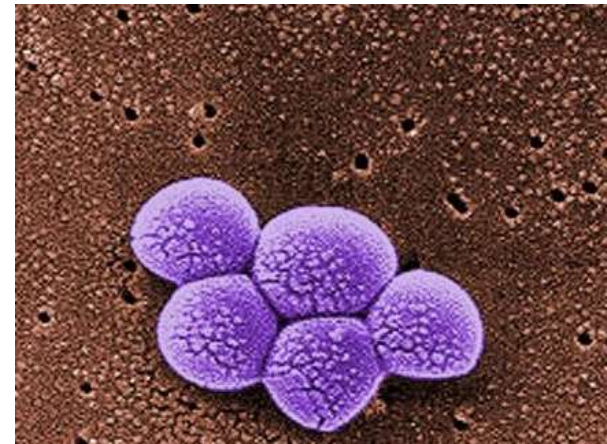
Fowlicidin-3

(FEBS J. 2007,
274: 418)



Fowlicidin-2

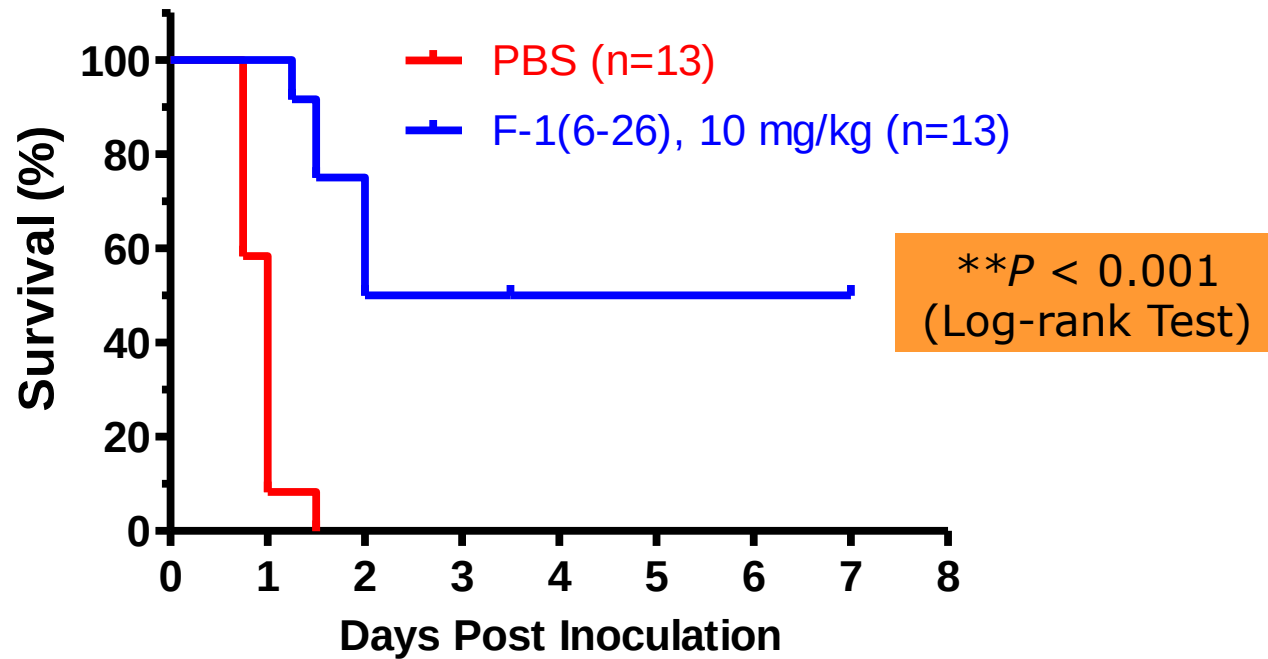
(J. Innate Immun.
2009, 1: 168)



**Direct delivery of fowlicidin-1 to
boost innate immunity against MRSA**

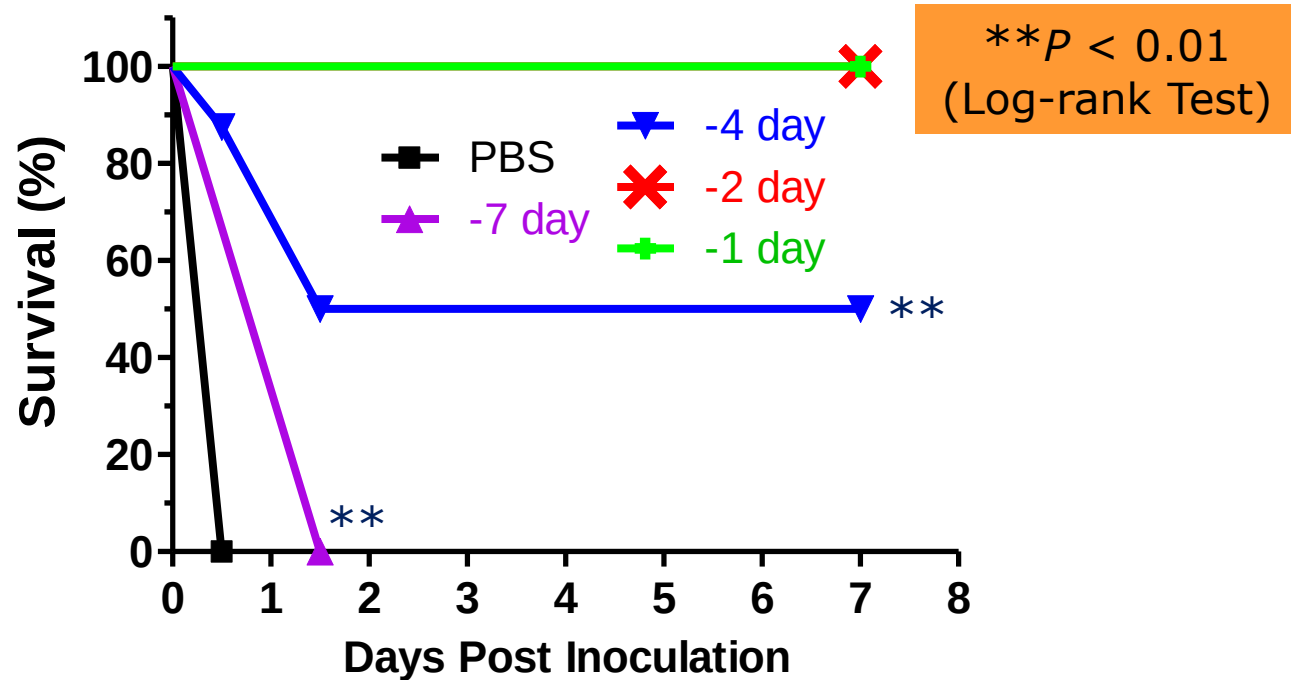


Protection of Mice From a Lethal MRSA Infection by Fowl-1(6-26)



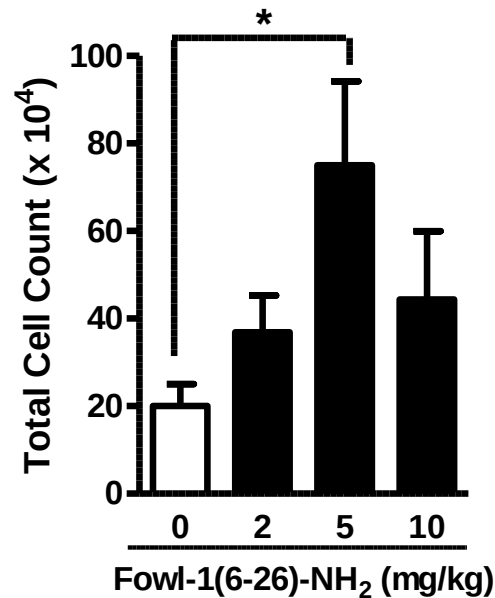
(Peptides 2010, 31: 1225)

Prevention of Mice From a Lethal MRSA Infection by Fowl-1(6-26)

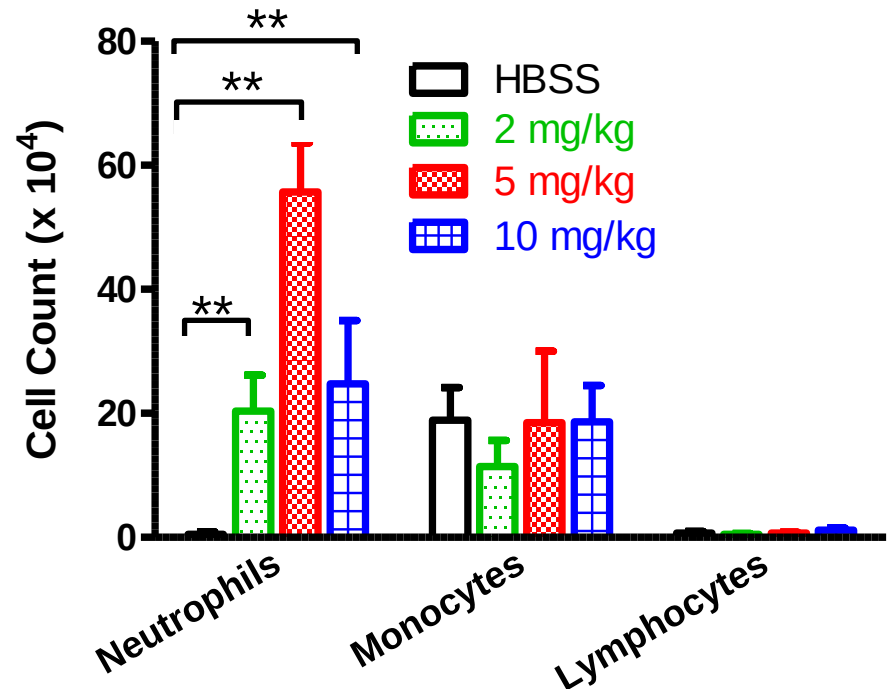


In Vivo Recruitment of Leukocytes by Fowl-1(6-26)

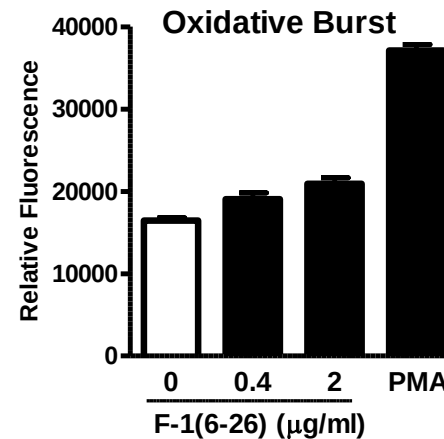
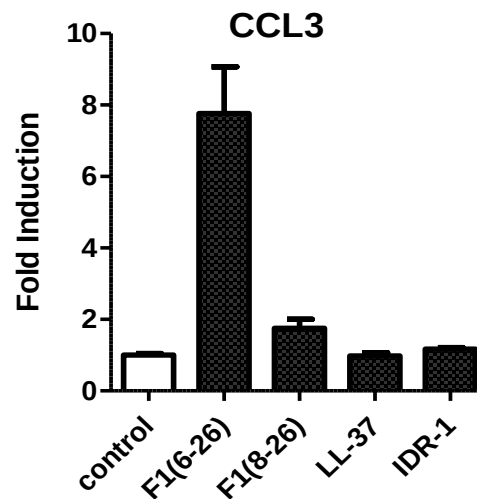
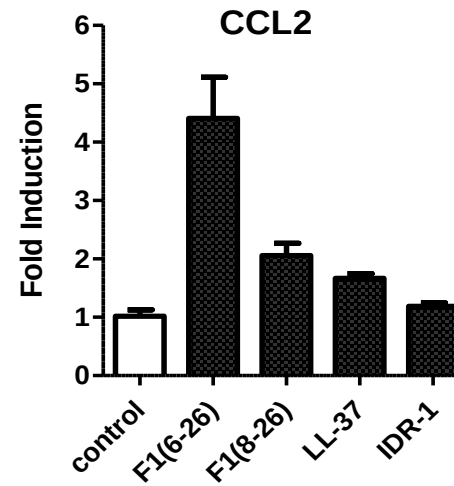
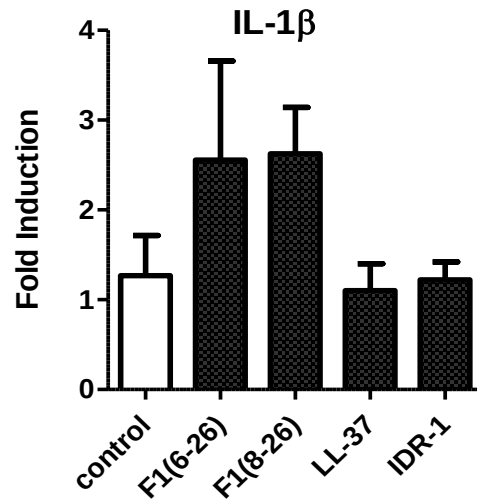
Total Leukocytes



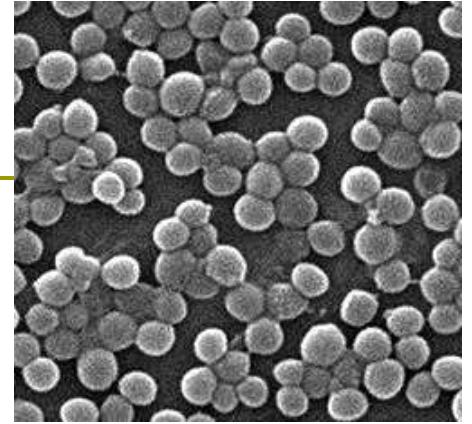
Leukocyte Subpopulations



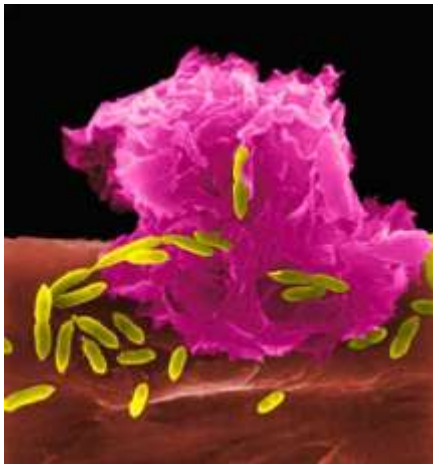
Activation of Macrophages by Fowl-1(6-26)



Summary



- Direct delivery of fowlicidin-1(6-26) is capable of treating and preventing mice from lethal MRSA infections;
- Prevention of MRSA infections by fowlicidin-1(6-26) is mediated in part by recruitment of neutrophils and activation of macrophages, but not by direct killing of bacteria;
- Fowlicidin-1(6-26) enhances surface expression of co-stimulatory molecules on macrophages and potentiates unpolarized antibody response following immunization with a model antigen (ovalbumin).

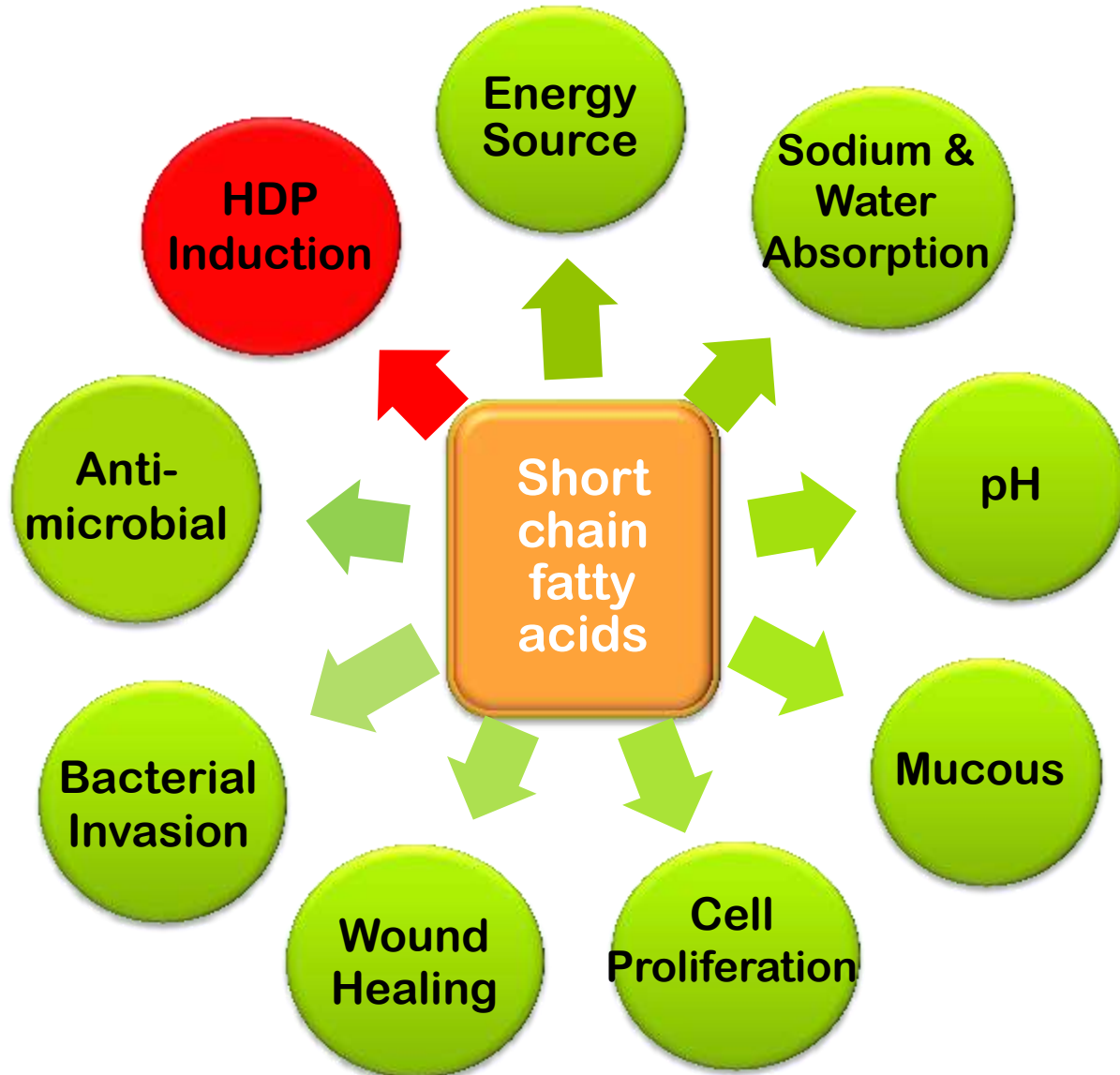




**Indirect dietary modulation of
endogenous HDP synthesis to boost
innate immunity against *Salmonella***



Physiological Roles of SCFAs



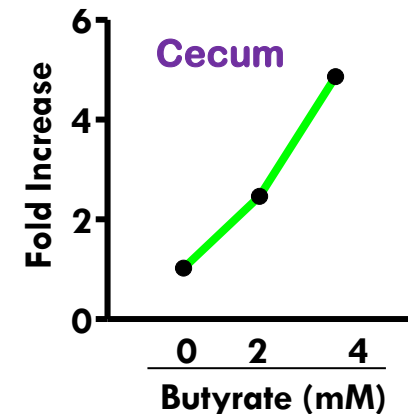
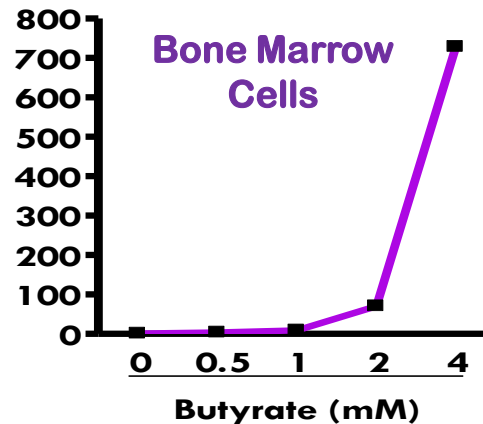
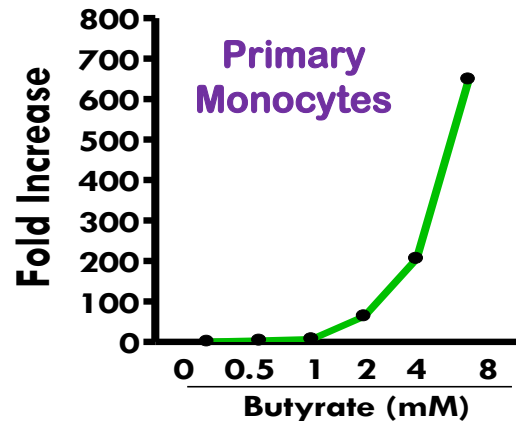
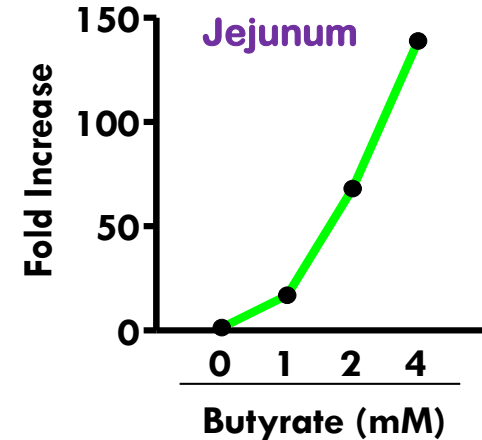
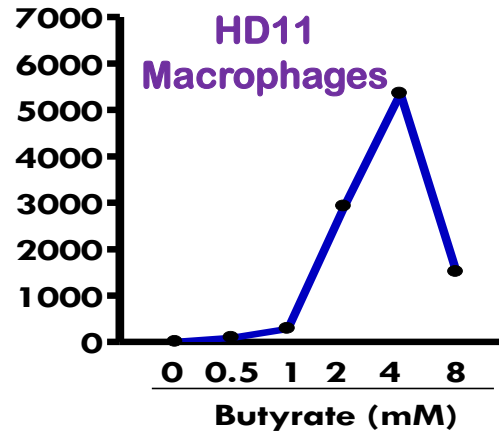
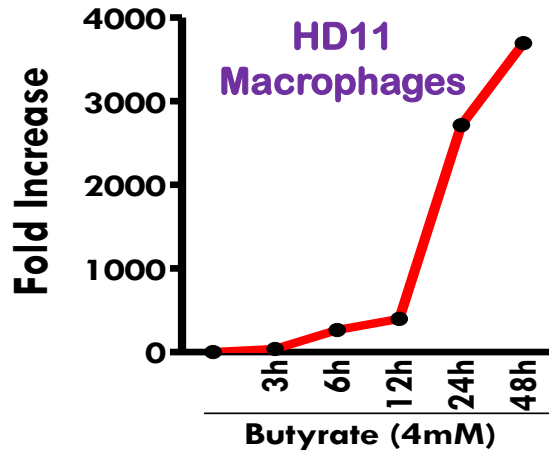
Butyrate Enhances Disease Resistance of Chickens by Inducing Antimicrobial Host Defense Peptide Gene Expression

Lakshmi T. Sunkara¹, Mallika Achanta¹, Nicole B. Schreiber¹, Yugendar R. Bommineni^{1a}, Gan Dai^{1b}, Weiyu Jiang¹, Susan Lamont², Hyun S. Lillehoj³, Ali Beker¹, Robert G. Teeter¹, Guolong Zhang^{1*}

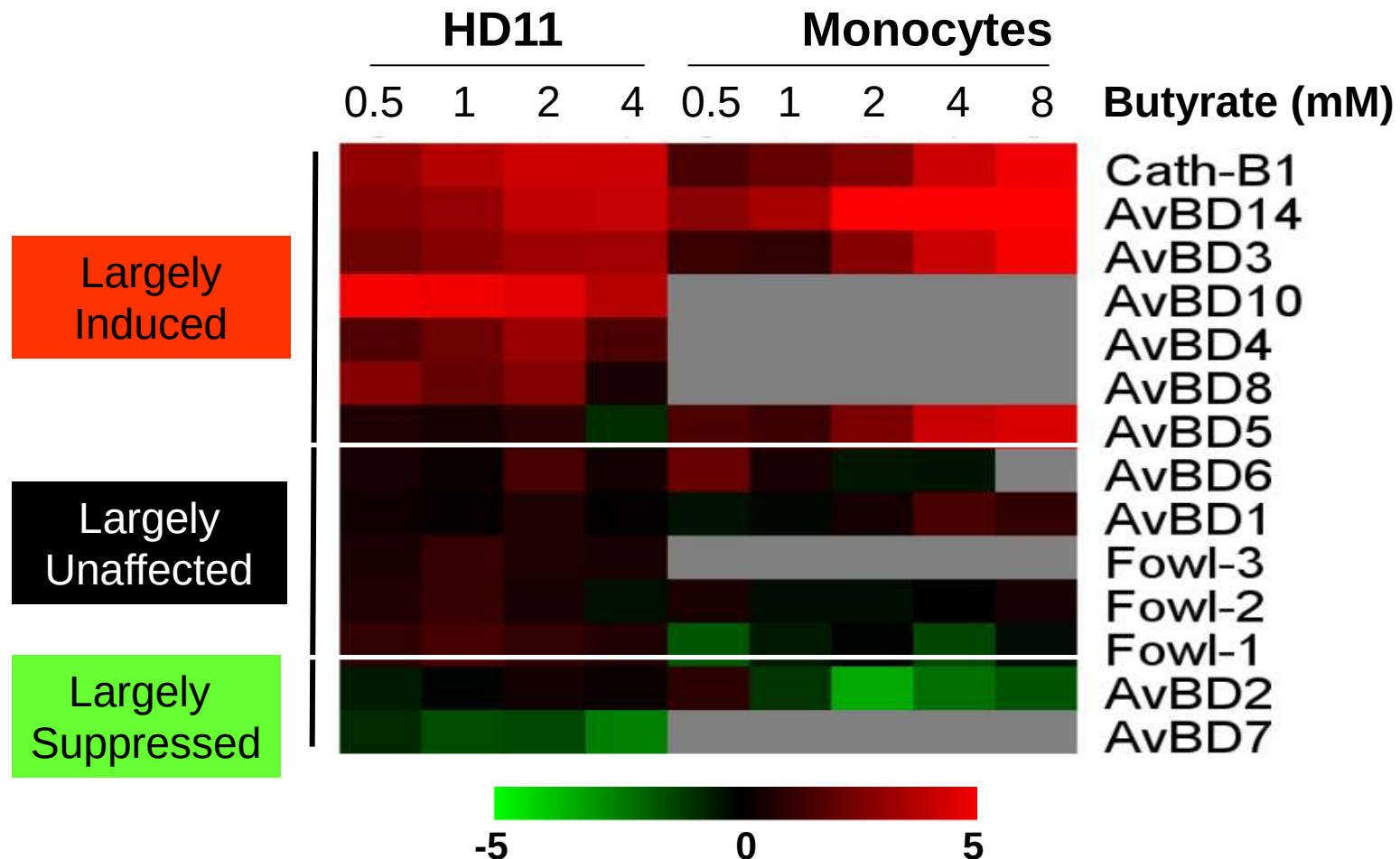
1 Department of Animal Science, Oklahoma State University, Stillwater, Oklahoma, United States of America, **2** Department of Animal Science, Iowa State University, Ames, Iowa, United States of America, **3** Animal Parasitic Diseases Laboratory, Animal and Natural Resources Institute, United States Department of Agriculture-Agricultural Research Service, Beltsville, Maryland, United States of America



Butyrate Induces Chicken β -Defensin 9 (AvBD9) Expression



Pattern of HDP Gene Induction by Butyrate



In vivo Induction of AvBD9 Expression by Butyrate

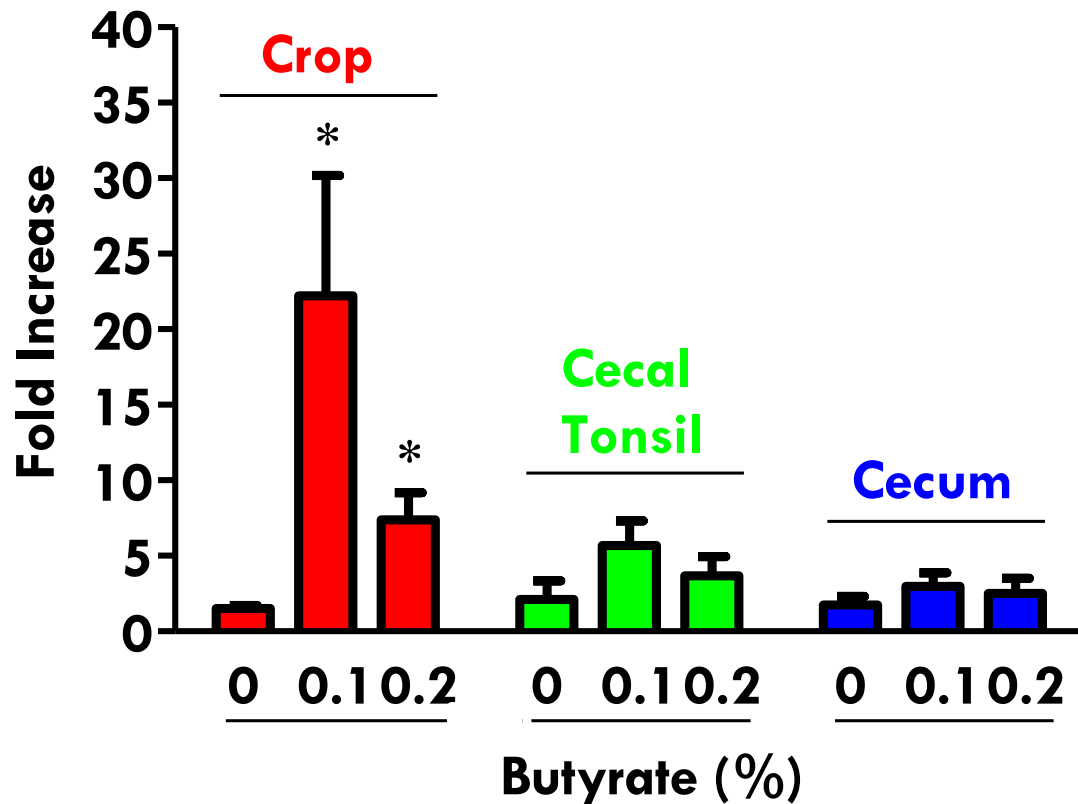


Figure 2: Effect of butyrate on *S. enteritidis* growth and colonization.

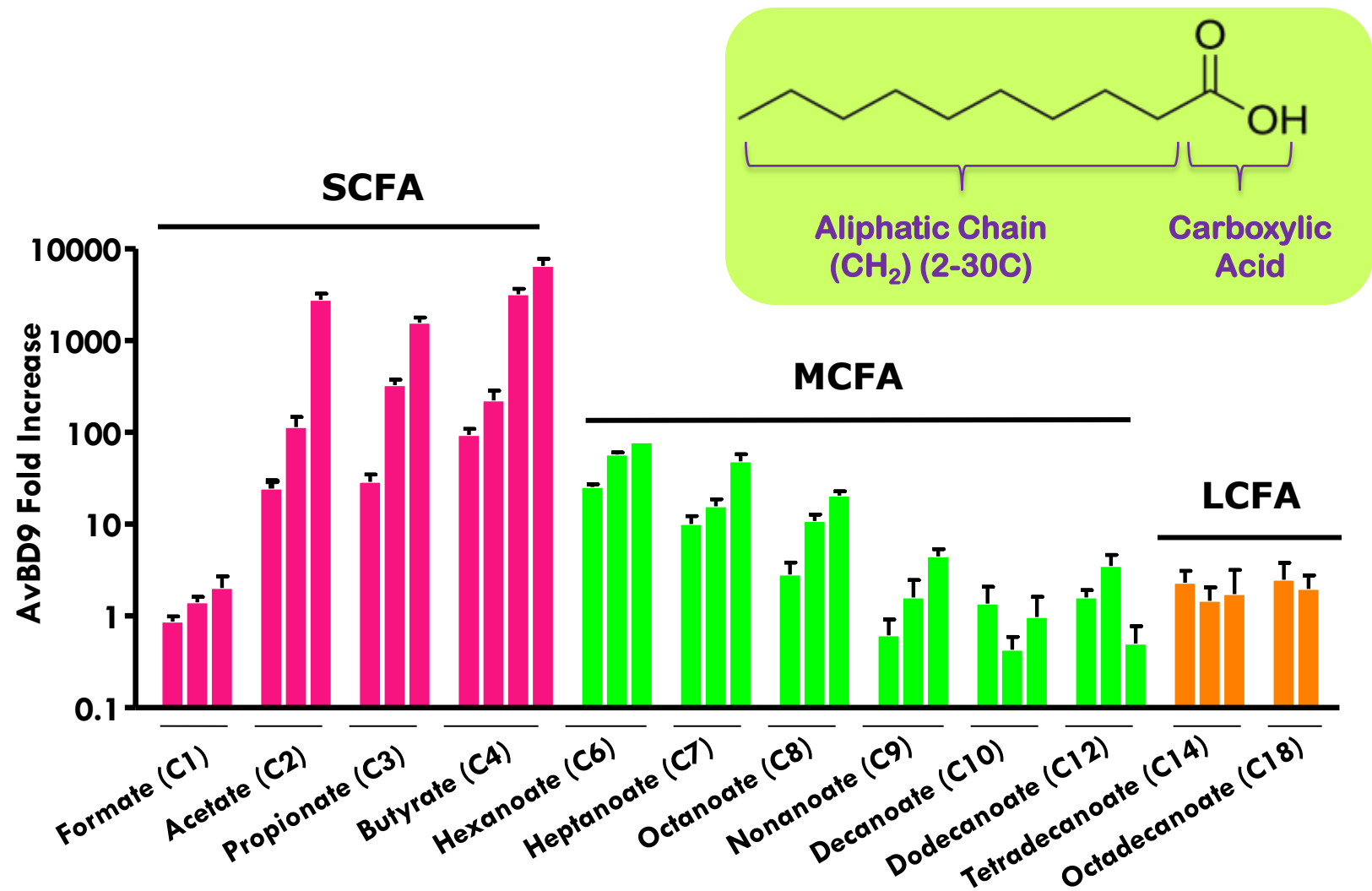
Left Panel: Scatter plot of *S. enteritidis* CFU/g. The y-axis is logarithmic, ranging from 10^5 to 10^9 . The x-axis shows three groups: Control (black circles), 0.1% Butyrate (black squares), and 0.2% Butyrate (black triangles). A horizontal line indicates the mean for each group. A bracket with $*P = 0.03$ indicates a significant difference between the Control and 0.1% Butyrate groups.

Group	CFU/g (Individual Data Points)	Mean CFU/g
Control	~5.5e7, ~5.5e7, ~8e7, ~1e8, ~4e7, ~5e7, ~6e6, ~7e6, ~8e6, ~9e6	~4e7
0.1% Butyrate	~1e5, ~2e5, ~3e5, ~4e5, ~1.5e6, ~2e6, ~3e6, ~4e6, ~5e6, ~4e7	~3e6
0.2% Butyrate	~3e6, ~3e6, ~2.5e7, ~3e7, ~4e7, ~5e7, ~4e6	~2.5e7

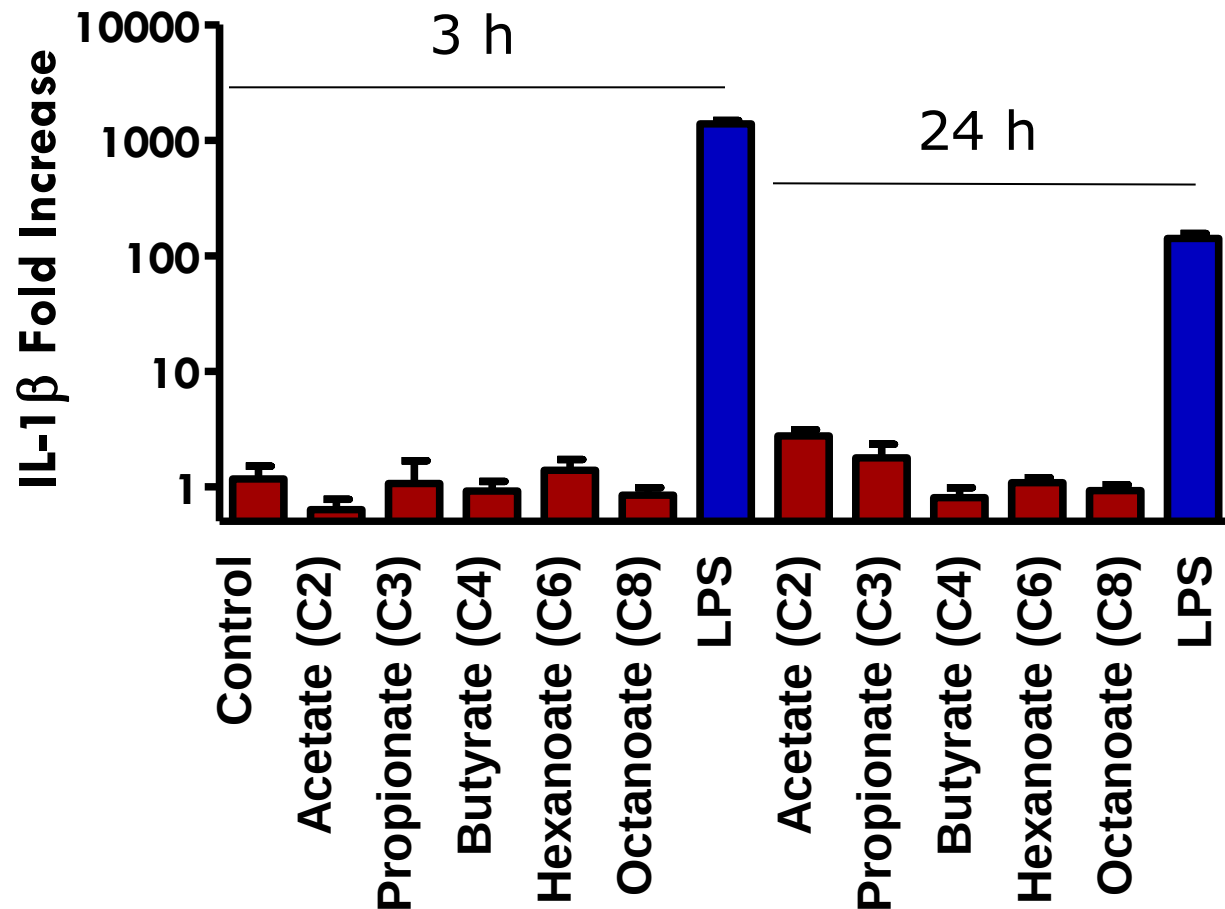
Right Panel: Bar chart of Fold Increase in *S. enteritidis*. The y-axis is linear, ranging from 0 to 40. The x-axis shows the fold increase for three tissues: Crop (red bars), Cecal Tonsil (green bars), and Cecum (blue bars) at 0, 0.1, and 0.2% butyrate. Error bars represent standard deviation. Asterisks (*) indicate significant differences (P < 0.05) for the Crop tissue at 0.1% and 0.2% butyrate compared to 0%.

Tissue	Butyrate (%)	Fold Increase
Crop	0	~1.5
	0.1	~22.5*
	0.2	~7.5*
Cecal Tonsil	0	~2.5
	0.1	~5.5
	0.2	~4.0
Cecum	0	~2.0
	0.1	~3.5
	0.2	~2.8

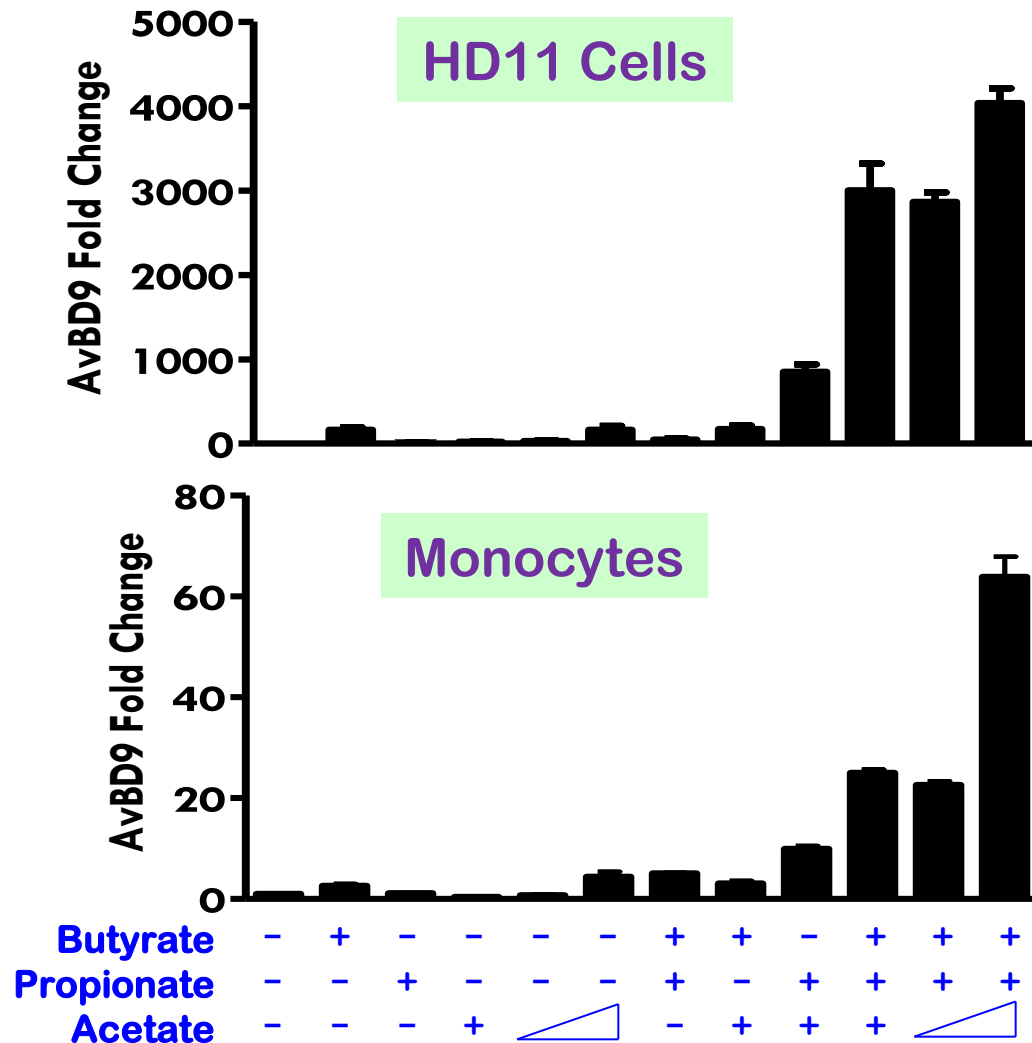
Correlation of Aliphatic Chain Length of Fatty Acids with HDP-Inducing Activity



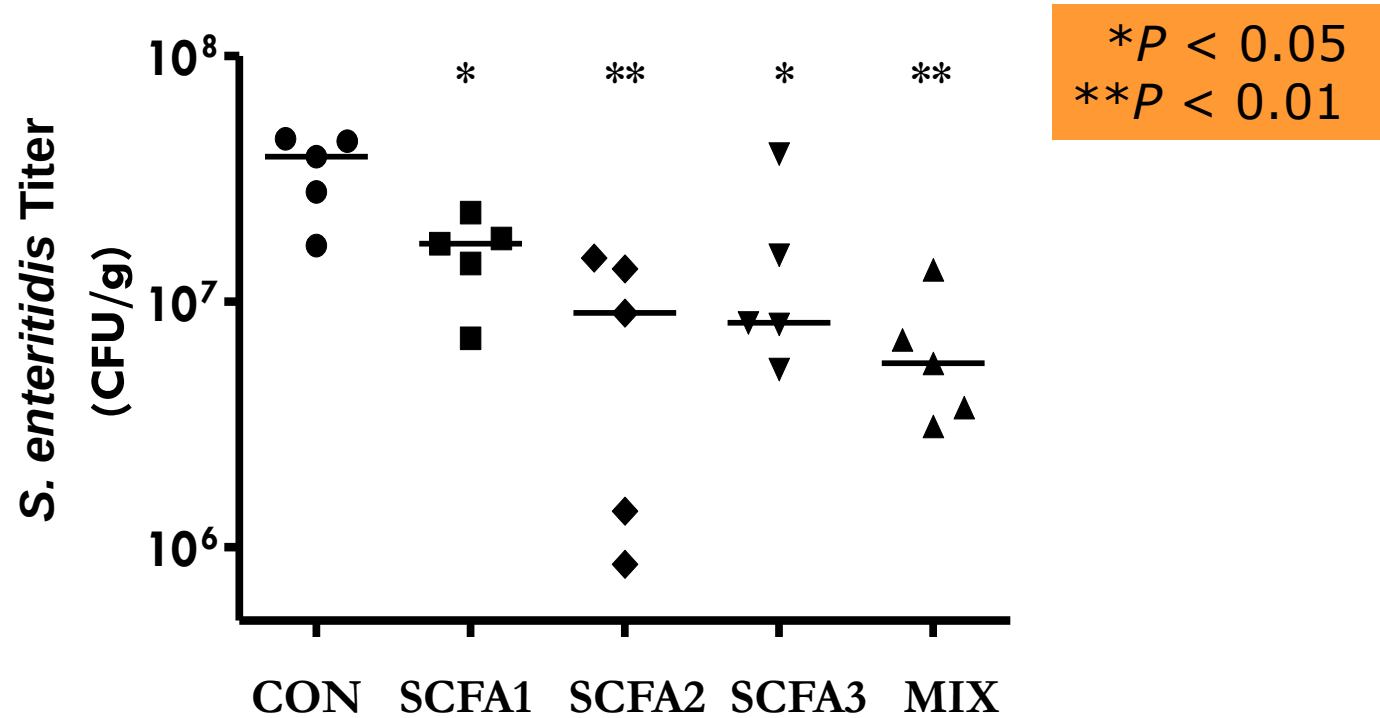
Minimum Impact of Fatty Acids on Proinflammatory Cytokine Expression



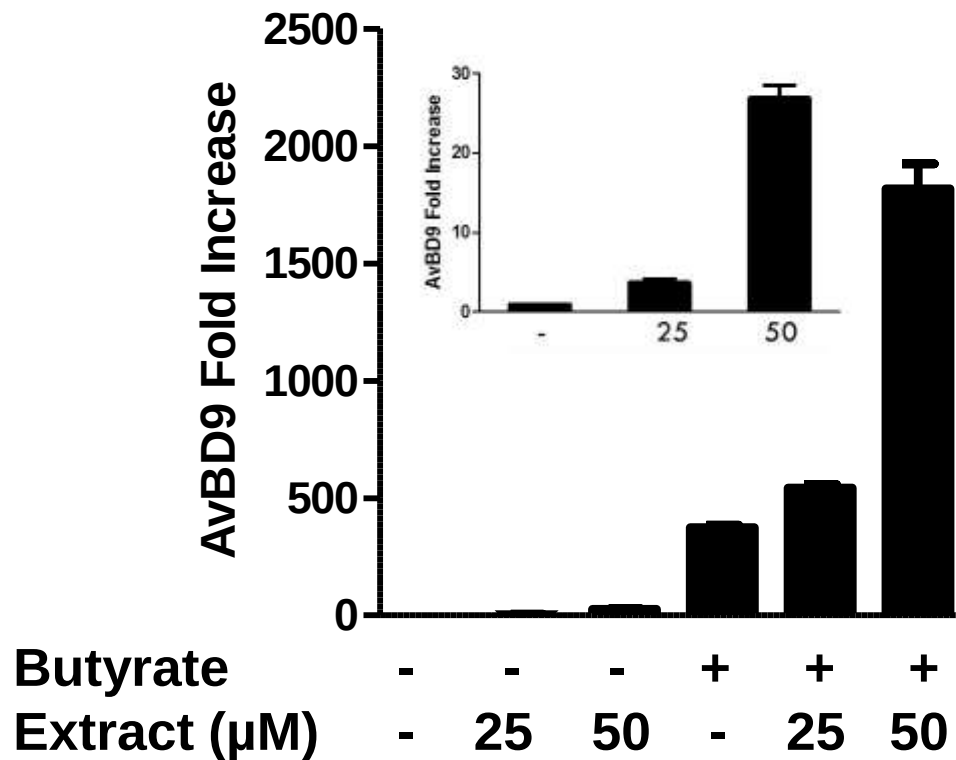
Synergistic AvBD9 Induction by SCFAs



Synergistic Reduction of *Salmonella* Colonization in Chickens by SCFAs



Synergistic Induction of *HDP* Expression Between Butyrate and a Plant Extract



Summary



- SCFAs are potent inducers of chicken HDPs both in vitro and in vivo by acting as histone deacetylase inhibitors;
- SCFAs synergistically enhance HDP synthesis and reduce *S. enteritidis* colonization with a minimum impact on proinflammatory response;
- A combination of natural SCFAs and herbal extracts may have potential to be explored as antibiotic alternatives;
- See a poster entitled "Development of immune boosting dietary supplements as alternatives to antibiotics" in Session 2 (2.11).

Funding



Oklahoma Center for the Advancement of Science and Technology



Oklahoma Agricultural Experiment Stations

Goal: Develop novel, highly efficient, cost-effective immune boosting agents in disease control and prevention for both farm animals and humans.