Research Project Summary

Student Name: [redacted] **Mentor Name:** [redacted]

Institution: University of Arkansas, Fayetteville **Classification:** Junior

Grade Point Average: 3.935 **Area of Study:** Animal Science

Title: Swine Enterotoxigenic *Escherichia coli* (ETEC) challenge from a disease and production standpoint.

The intended purpose of this study is to gain an understanding, from a research perspective, of how pathogenic *Escherichia coli* affects growth and development during the early weaning period of the swine and determine the effectiveness of dietary fiber source and fiber degrading enzyme on the sustainability of ETEC challenge.

Colibacillosis is the leading cause of post-weaning diarrhea in food-producing pigs. Current strategies for the prevention and treatment of porcine diarrhea include vaccination, antimicrobial therapy, and pharmaceutical levels of zinc. However, concerning the efficacy of vaccination, antibiotic resistance, and environmental pollution urge the researchers to evaluate environmentally friendly alternative strategies.

I will examine whether dietary fiber and fiber degrading enzymes introduced to swine will modulate their gut microbiome during periods of clinical illness. Dietary fiber has been shown to promote a healthy GI tract bacteria community, while fiber-degrading enzymes are suggested to increase the benefits of carbohydrates and oligosaccharides; which help to promote host health by alleviating deleterious effects of proinflammatory responses. I hypothesize that dietary fiber and fiber-degrading enzymes can help tone the microbiome when pigs are challenged with ETEC, boost growth performance, and generate a synergistic effect.

Background and Significance

Escherichia Coli was first isolated by German pediatrician Theodor Escherich in 1884 [4]. Because of its strong survivability and specific characteristics, it has been used as a model in biomedical research to elicit the mechanisms of gram-negative bacteria and a vehicle to deliver the treatment [1].

The commensal strain *of E. coli* is one of the earliest colonizers in the gastrointestinal tract and is a core microbiome. Early colonization of commensal *E. coli* plays a key role on anaerobic bacteria colonization and immune development in neonates. Despite the nonpathogenic strain virulence, gene-encoded strains have been shown as one leading causes of bacterial infections in both humans and animals [8].

Colibacillosis is a common, but detrimental, occurrence in different production stages of swine including suckling, and post-weaning period. Contracting ETEC can lead to pecuniary loss as a result of mortality, morbidity, decreased growth rate, and an increase in the cost of medication[5]. During suckling, antibodies are passed from the sow to the piglet postnatally through colostrum and milk to help neonates damp the detrimental effects of infectious. This protection, however, is no longer available once weaned. Piglets' immune systems at this age (21 days) are less regulated and the microbiome is still at the plasticity stage which can result in dramatic responses and increased susceptibility to pathogens that contribute to the severity of this disease [5].

Dietary fiber, a carbohydrate compound that is indigestible in the foregut or pigs, is the source of nutrients for beneficial microbes [11]. Thus, it nurtures the multiplying of probiotics to help control pathogenic strains to take over the GI tract [3]. The United States Department of

Agriculture recommends 14 grams per 1,000 calories of food intake for people as metabolites produced from these probiotics have also been shown to prevent and control clinical diseases [11].

Upon weaning, pigs' diets change from milk to solid plant diets. This presents a chance for increased stress on the underdeveloped digestive system. The excess undigested nutrient influx into the hindgut can disturb the balance of the community and induce inflammatory responses [7]. Therefore, fiber-degrading enzymes are suggested to be used to overcome potential detrimental effects. The mode of action on fiber degrading enzymes comprises the reduction of complexity of plant fiber to improve the efficacy of digestive enzymes on increase the nutrient bioavailability and the increase fiber accessibility to bacteria, which increases the production of oligosaccharides and health-beneficial metabolites [6].

Antibiotic usage in swine production increases the development of antibiotic resistance to pathogens, including ETEC. The recent outbreak of antibiotic-resistant fimbriae 18 (F18) expressed ETEC is an alert to producers to reconsider medication usage.

Objectives and Hypothesis

The objective of this proposal is to determine the consequences and outcomes of dietary fiber and fiber-degrading enzymes on *E. coli* populations within the swine gastrointestinal microbiome and the effect and progression of ETEC during the infection period. I hypothesize that enzyme and fiber treatments can modulate the microbiome and help pigs sustain the ETEC challenge which will ultimately promote their growth performance.

Methods

Research Design. To measure clinical illness and stress in piglets across different treatments, I will select a total of 216 piglets and block them by body weight where they will then be allotted to nursery pens. Pens within blocks, will then randomly be assigned to 1 of 4

dietary treatments. There will be 8 replicates (pens) per treatment group with 6 pigs/pen, and 36 pens. In this case, one median body weight pig from each pen will be selected for fecal sampling, and the same pigs will be sampled repeatedly in the remain of study. This will allow for observation of the responses of each pig in the ETEC challenge. All pens are intended to be kept as gender neutral with 3 barrows and 3 gilts. All piglets are to be inoculated with the F18 strain of *E. coli* 5 days postweaning.

Data and samples collections. Individual pig body weight and pen intake data will be recorded during the study to determine the body weight, average daily gain, average daily feed intake, and feed efficiency. Fecal samples will be collected prior to ETEC administration, and again on day 2 and day 7 post-challenge to quantify and characterize the *E coli*.

Treatments. Dietary fiber and fiber-degrading enzyme will be provided from weaning (day 0) to day 22 and then a common diet will be fed from day 22 to the end of the trial. Dietary treatments will be high fiber diet (HF), high fiber and fiber degrading enzyme (FS), control diet and fiber degrading enzyme (CS), and control diet (CC).

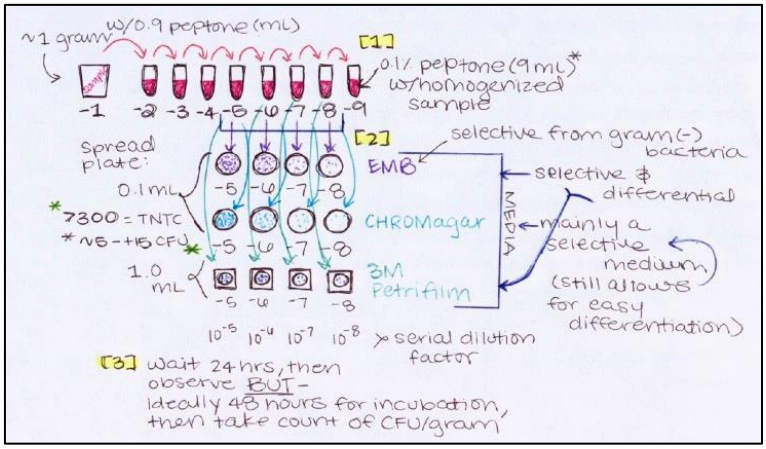
Serial Dilution Factor Determination. A 1-gram fecal sample is to be placed into a 15 mL culture tube along with 9 mL of a diluent, 0.1% peptone diluent. The use of 0.1% peptone during dilution further maximizes the avoidance of the destruction of bacteria during the process [10]. Additionally, bacterial losses can contribute to large errors in quantitative determination of the CFU/gram (colony forming units per gram). Samples will then be placed in a stomacher to homogenize prior to dilution and plating.

Eight other culture tubes containing 9 mL of 0.1% peptone are to be labeled as 10^{-2} to 10^{-9} (fecal sample is considered 10^{-1}) and then placed in a biosafety cabinet. For the test run and dilution process, I will pipette 1 mL of the fecal sample into 9 mL of 0.1% peptone, thus

increasing the dilution factor for each subsequent culture tube. When accounting for the F18 strains of *E. coli* introduced to by gavage to the pigs, I will utilize a serial dilution factor of 10^{-4} to 10^{-7} plating of the suspension.

Figure 1

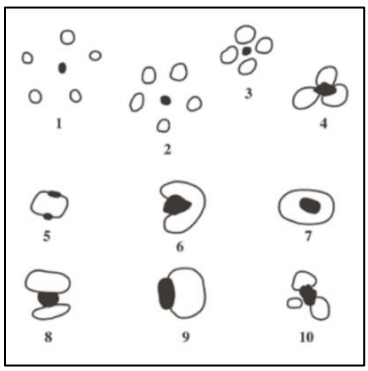
Serial Dilution Determination Factor



Note. The dilution factors of 10^{-5}, 10^{-6}, 10^{-7}, and 10^{-8} were determined to yield a reliable CFU/gram count. Created by author.

Measurements. I will detect and enumerate *E. coli* coliforms by use of 3M Petrifilm. Coliforms produce acid and gas through lactose fermenting coliforms, and most *E. coli* colonies will appear as blue to red-blue colonies [12]. I will not count blue colonies without surrounding gas bubbles as *E. coli*. Other coliform colonies present a reddish-pink hue and are closely associated with entrapped gas. This study will utilize a total coliform count which consists of both the red and blue colonies with gas and the *E. coli* count (blue colonies with gas). After picking, colonies will then be isolated in nutrient broth.

Figure 2



Note. Illustrations depicting various bubble patterns indicative of gas-producing colonies (coliforms). All should be enumerated and accounted for. From "3M™ Petrifilm™ *E. coli*/Coliform Count Plate", by 3M Food Safety, 2017, pg. 2. Copyright 2017 by 3M.

Quantification. A polymerase chain reaction (PCR) assay will be utilized for the detection and characterization of pathogenic *E. coli*[2]. From there, I will be able to identify the genotypic characterization of *E. coli* isolates from pigs experiencing post-weaning diarrhea (PWD).

Table 1

DNA sequence of polymerase chain reaction primers for fimbrial and toxin genes.

Virulence factor (gene)	Primer sequence	Melting temperature (°C)	Product size	Accession no.	Reference
STb	TGCCTATGCATCTACACAAT	58	113	M35586	22
(*estB*)	CTCCAGCAGTACCATCTCTA	59			
STaP	CAACTGAATCACTTGACTCTT	57	158	V00612	35
(*estA*)	TTAATAACATCCAGCACAGG	57			
K99	AATACTTGTTCAGGGAGAAA	55	230	X05797	34
(*fanA*)	AACTTTGTGGTTAACTTCCT	56			
LTb subunit	GGCGTTACTATCCTCTCTAT	57	272	M17873	9
(*eltB*)	TGGTCTCGGTCAGATATGT	59			
F18	TGGTAACGTATCAGCAACTA	58	313	M61713	20
(*fedA*)	ACTTACAGTGCTATTCGACG	59			
987P	AAGTTACTGCCAGTCTATGC	59	409	M35257	10
(*fasA*)	GTAACTCCACCGTTTGTATC	57			
K88	GTTGGTACAGGTCTTAATGG	57	499	M35954	21
(*faeG*)	GAATCTGTCCGAGAATATCA	56			
F41	AGTATCTGGTTCAGTGATGG	58	612	M21788	2
(*fedA* subunit)	CCACTATAAGAGGTTGAAGC	57			
Stx2e	AATAGTATACGGACAGCGAT	57	733	M21534	37
(A subunit)	TCTGACATTCTGGTTGACTC	58			

Note. By T. A. Casey and B. T. Bosworth[2].

Anticipated Outcomes. I anticipate that ETEC bacteria will disturb the balance of the gut microbiome, decrease *E. coli* diversity, and contribute to PWD. I posit that dietary fiber and fiber-degrading enzymes will modulate the bacteria community and help overcome the deleterious effects of ETEC. The outcome of this research can provide the swine industry with alternative options in dealing with the incidence of colibacillosis.

Timeline (2024)

Jauary-February	• Validation of research design • Material inquiry
March-May	• Live animal experiment • Live animal data analysis • Attend ASAS Midwest Conference
August-September	• *E. coli* count/quantification • Continued enrollment
October-November	• Begin and complete polyemerase chain reaction (PCR) • Begin and complete gene sequencing and virulence identification
December	• Write paper • Report project data/poster

Literature Cited

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