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Evaluation of Maternal Diet and its Effect on Milk Composition and Piglet Health and Growth Performance

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EVALUATION OF MATERNAL DIET AND ITS EFFECT ON MILK COMPOSITION AND PIGLET HEALTH AND GROWTH PERFORMANCE

by

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Graduate research and graduate teaching duties work together to develop a graduate student's skills both in the classroom and on their research experiments. Being a GTA and GRA allows a student to form more sound hypotheses, connect better with students, and better understand their own research.

During the time as a GTA and GRA four surveys were developed to analyze different groups of students and their learning environment and two animal experiments were conducted to evaluate maternal diet and its effect on milk composition and piglet health and growth performance

Surveys given to students consisted of multiple choice, fill in the blank, and Likert scale questions. Surveys were taken anonymously, and no revealing information was asked. Upon completion of each survey, they were analyzed. Improvements and strong points among each topic were noted and discussed. Survey topics included in analysis were the use of case studies in vet school, an assessment of the animal science department through an animal science senior survey, why students chose the animal science major, and the evaluation of undergraduate research at UNL.

The animal research that was conducted was done on 2 separate batches of sows. The first experiment consisted of batch 16 parity 1 sows. Sows were fed either 1) Control diet formulated to NRC (2012) specifications (CTL); 2) Restricted (20% energy restriction via addition of 40% soy hulls; RESTR); and 3) Control diet plus addition of crystalline amino acids equivalent to the SID Lys:ME of the RESTR diet (CTL+). during the gilt development stage of days 123-240. Diet may be correlated with milk peptide composition and fecal microbiome of the piglet.

The second experiment focused on batch 17 parity 4 sows (n = 30). Sows were all on a common gestation diet except 10 had the recommended value of a probiotic toped dressed on their feed, 10 were a control, and 10 had 5% more than recommended value of the probiotic top dressed on their feed. The top dressing was started on day 80 and continued until farrowing. Sow diet during gestation may affect the milk composition and piglet microbiome and piglet performance.

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Introduction

As a PhD student at the University of Nebraska-Lincoln in the Animal Science Department, my program was structured somewhat differently than other animal science students. My PhD program was divided into two areas which included animal research experiments and teaching survey experiments. I was co-advised by two professors in which one oversaw all my animal research and the other oversaw all my teaching research. On top of conducting research in two separate areas, I also invested in coursework that aligned with my projects. Not only did I take Animal Science classes through the duration of my program, but I also took many classes to help teach me how to write proper surveys and how to become a better teacher. This division was of great interest to me because it allowed me to expand my knowledge in more ways than one. As a master's student, I solely focused on animal research; however, during my PhD I was able to focus on another aspect and strengthen my skills as a teaching assistant and researcher.

Benefits of Duel Graduate Teaching/Research Assistantships

In today's universities, it is common practice to employ graduate students as graduate teaching assistants (GTAs) (Reeves et al., 2016). Not only does the university benefit from the use of GTAs, but so do the graduate students as they are typically offered financial support and it aids in their professional development (Gilmore et al., 2014). Furthermore, due to closeness in age, undergraduates may feel more comfortable approaching a GTA and tend to have a more personal relationship with them (Reeves et

al., 2016). In a study conducted by Kendall and Schussler (2012), students' perceptions of GTAs and professors were compared in the teaching of biology courses. Results showed undergraduates identified their professors as more structured, confident, in control, organized, experienced, knowledgeable and respected, but on the contrary they were also more distant, formal, strict, serious, boring and out of touch. Yet, GTAs were perceived as relaxed, interactive, understanding, and able to personalize teaching, but uncertain, hesitant and nervous. While many GTAs are teaching undergraduate students they typically do not have prior training before doing so; thus, there are several items that have been shown to enhance the efficacy of GTA teaching proficiency. It has been demonstrated that prior research experiences may be pivotal in establishing teaching practices or abilities in the classroom (Windschitl, 2003). However, it is still undetermined the time and depth of research experience needed for GTAs to enhance students' understanding of science, technology, engineering, and mathematics (Feldman et al., 2009). In a study conducted by Feldon et al. (2011), it was concluded that students that engage in both research and teaching are more able to generate testable hypotheses and sound research experiments when compared to students who focus solely on research. Furthermore, teaching experience can contribute greatly to one's essential research skills (Feldon et al., 2011). When teaching in the same field as your research program, a GTA is required to explain or review topics similar to their research and guide their students in learning. A possible benefit of this approach is that a GTA is then further reinforcing their own learning. Typically, assistantships focused mainly on research do not require constant explanation of a topic resulting in the possibility of information and concepts being less understood or not retained at all (Feldon et al., 2011). While being a

GTA may help enhance understanding of research, reports have also shown the graduate students having both research and teaching responsibilities results in more conference presentations and higher publication rates (Ethington et al., 1993). Through my personal experience in roles including teaching and research, I have experienced greater publication rates, enhanced critical thinking and problem-solving skills. The following literature review will discuss topics of my animal research that were done congruently with my teaching assistantship.

Introduction to the Effects of the Maternal Diet on Progeny Health & Growth

The United States is the third largest producer and consumer of pork and pork products and fluctuates between being the largest and second largest exporter of pork and pork products (USDA, 2019). According to PigChamp 2019 (U.S. 3rd quarter summary, 2019), anywhere from 14-15% of a sow's litter dies pre-weaning. Furthermore, up to 17% of those deaths can be attributed to insufficient milk (Alonso-Spilsbury et al., 2007). Thus, in a 1000 head sow operation averaging 2.6 litters per year, a pork producer can decrease its mortality rate by about 676 piglets per year by increasing maternal milk output/nutritional value. Then, if all these pigs can be marketed after weaning, a producer can earn an extra $30,000/year. The diet in a breeding herd can greatly influence sow productivity and longevity of the herd through increasing milk output and milk nutrient composition. On a bigger scale, Lonsinger (2005), reported that a decrease in the mortality rate for suckling pigs would cause an increase in pork production and, assuming no suckling pigs had died during 2005, the total gain to the US economy would have been $250 ± 30 million. Most costs, including feeding costs, in the breeding herd are

fixed costs and therefore, increased breeding herd efficiency will reduce overall production costs.

Pork producers today continue to focus on increasing piglet weight while keeping the price of production down. As pork producers continue to focus on longevity and increased litter size, nutrition of the sow is becoming more important. Diet of the sow has been shown to greatly impact the nutrient composition of milk (Amdi et al., 2013). As nutrient composition of the milk increases, there should be a direct correlation to piglet health and growth. Throughout lactation, there are varying nutrients that all have specific effects on the piglet such as neural development and immune response. The suckling piglet not only gets immediate benefits from the milk of the lactating sow but there are also long-term effects on both health and growth performance (Aherne, 2019).

With the high interest in increasing weaning weight while simultaneously keeping litter numbers high, nutritionists continue alter sow diets to fit the needs of neonate piglets and improve milk nutrients of the lactating sow. One way to do this successfully could be through nutritional changes in the gestating sow's diet. Dietary interventions of the sow have effects on milk that can attribute to health and weight development of their piglets (Barnett et al., 2017). Sow diets have been shown to have specific effects on the composition and nutrient availability in milk (Barnett et al., 2017). Milk composition is sensitive to both the diet and environment of the sow (Hurley, 1997). Through changing the diet of the sow, piglets will also then have an altered microbiome similar to that of the sow's (Laskowska et al., 2019). Specific components of milk have a direct effect on the piglets' microbiome and its ability to fight off unwanted pathogens and increase growth performance (Laskowska et al., 2019). Furthermore, through nutritional intervention of

the sow, pork producers may be able to increase litter size while also lowering mortality and morbidity rates.

Therefore, the focus of this review to explain the importance of maternal nutrition for sows to consistently produce high quality piglets that make it to weaning.

Altering Milk Profile

As sow litter size increases, there is a decrease in piglet birthweight and subsequently piglet vitality and growth (Vanden Hole et al., 2018). Most research today focuses on feeding regimens of the piglet and how to directly impact their weight; consequently, very few studies look at altering the lactation and gestation diets of the sow to increase milk nutrient value especially since there is an increase in littler sizes (Declerck et al., 2016; Schmitt, 2019). With sow productivity drastically increasing over the past two decades, sow energy requirements need to be re-evaluated. Sows are being selected for larger litter sizes; however, little research has been done to address the new nutrient requirements of the sow to support these larger litters. While increasing the energy of a lactation diet may increase the growth rate of the nursing piglets (Choi et al., 2017), one must also look into supporting the health and longevity of the sow because if she becomes deficient her piglets will not thrive.

During gestation, nutrition is the main environmental factor influencing the development of the embryo (Costa et al., 2019). Maternal microbiota influences the offspring's gut microbiome through direct contact with the sow and the ingestion of milk, and this then contributes to the overall health of the offspring (Gomez de Aguero et al., 2016). Altering the diet of the sow may be an effective way to improve neonate immunity and growth (Shang et al., 2019). Maternal milk is a complex fluid that not only supports

growth and development of infants, but also help enhance their immune functions, microbial diversity, and hormones which are all needed to aid the body in adequate homeostasis (Grabarics et al., 2017; Ballard and Morrow, 2013). As the offspring gets older the breastmilk changes in composition from colostrum to late lactation to fit the needs of the infant (Ballard and Morrow, 2013). Within the changes of milk over time there are thousands of distinct bioactive molecules that help the infant protect itself against infection and inflammation as it matures (Ballard and Morrow, 2013).

Maternal milk nutrients are derived from 3 sources which include synthesis in the lactocyte, diet, and maternal stores. The maternal diet greatly influences the composition of fatty acids in the milk, particularly oleic acid and linoleic acid (Innis, 2014; Koletzko, 2016). Furthermore, while breastmilk is highly conserved in the body, maternal diet is important in various vitamins and fatty acid composition of the milk to meet the nutrient needs of the infant (Valentine and Wagner, 2013). There are numerous nutrients within breastmilk that play a key role in an infant's neurological development. Vitamin A, B6, B12, and folate, as well as, iodine and selenium help with neurological development and these nutrients vary greatly within breastmilk depending on the maternal diet (Ballard and Marrow, 2013). All these vitamins are necessary among all mammals yet in difference levels or for different reasons due to different diets and metabolism. Vitamin A is more likely to be deficient in humans because of the foods they are recommended to avoid during pregnancy; however, because of a formulated diet specific for gestating sows, this deficiency typically does not occur in sows. Due to swine having increased synthesis of new tissues during gestation, and large litters, they have an increased demand for nutrients compared to non-litter-bearing mammals. With high demand consistent with

maintaining larger litters, both in quantity and quality, there is an increased demand for b-complex vitamins during gestation. Having the optimal amount of b-vitamins (which is not clearly established) results in maximized metabolic status and growth (Matte et al., 2006). While an increase in Selenium in humans has shown an increased in birthweight and neurological affects, in pregnant sows it has been shown increased litter size and birthweight (Pinelli-Saavedra, 2003). Vitamin D, while used in calcium and phosphorus homeostasis in all mammals, vitamin D specifically in pigs is used for calcium metabolism and to promote fetal growth (Halloran, 1979).

Lipids make up the second largest fraction of breastmilk and provide the infant with energy (Koletzko et al., 2001). Milk lipids are typically triacylglycerols within fat globules that are formed in the mammary gland from fatty acids. Increased offspring growth is likely attained by increased fat and lactose concentration present in the milk (Kim et al., 2018). This is consistent among all mammals, including the pig; however, there are some differences in pigs when compared to humans. These differences include feeding sows differently based on how many litters they have had and altering diets to decrease backfat loss during gestation and lactation. Sow milk is extremely high in fat containing around 8%, whereas humans is 4.5% yet this will vary based on diet (Hurley, 1997). The pig and human have many of the same requirements, yet with altered levels due to number of offspring, age, and species and purpose of reproduction.

There are also studies on the modification of breastmilk through maternal immunization. Trials of maternal immunization have shown significant increases in immunoglobulins present in the milk (Steinhoff and Omer, 2012). Furthermore, in human

breastfeeding, because the maternal diet is not always optimal, multivitamins are recommended during lactation to help enhance milk output quality (Allen, 2012).

In conclusion, diet of the sow plays a key role in the milk nutrient profile. Altering diet and focusing on the needs of a lactating sow can provide better health and growth of offspring

Microbiome

Gut microbiota play an important role in the immune system of an animal during development (Carney-Hinkle et al., 2014). Understanding the gut microbiome of the pig can help increase its health by populating the gut with beneficial bacteria and ones that fight off pathogens. The objective of this section is to focus on the various components that can affect the piglet microbiome including, maternal diet, environment, and formula vs. maternal milk.

The gut is full of millions of bacteria that contribute to the microbiome. The microbiome of any mammal is very specific to that individual and has a profound effect on host health and weight (Graf et al., 2015). The adult gastrointestinal tract (GIT) is said to inhabit 400-500 species of bacteria but can get to be over 800 species (Graf et al., 2015)). Results show when pro- and prebiotics are consumed, gut microbiota is altered and there is a greater development in intestinal immunity of the offspring (Laskowska et al., 2019).

The interactions between host, diet, and microbiota become very pronounced during the postnatal phase as GIT determines the amount, species, and diversity of bacteria that will establish within (Buddington and Sangild, 2011). Nonruminant animals such as the pig, have a decreased density and diversity of bacteria in their stomach vs

other gut regions, due to the high acidic component in the stomach (Buddington and Sangild, 2011). Microbial populations are present throughout the entire gut, the oral cavity to the rectum; however, the density and composition of microbes varies based on site as well as on environment, transit rates, substrate availability, and the gut wall (Graf et al., 2015). The stomach, due to its high pH and oxygen exposure have low numbers of microorganisms compared to that of the large intestine. Based on microbiome analysis, the majority of bacteria that populate the gut are Bacteroidetes and Firmicutes. Bacteroidetes are the major producers of propionate and are able to produce a large variety of other substrates (El-Kaoutari, 2013) while Firmicutes, which include proteobacteria, are the major producers of butyrate and focus on degrading indigestible polysaccharides (Louis et al., 2010). Firmicutes and usually less abundant in a healthy gut.

Sterile compartments within animals, such as the prenatal gut, have an impaired immune system and colonization of gut microbiota through nursing and environmental factors helps to enhance the immune system (Round and Mazmanian 2009). Furthermore, different immunoglobulin concentrations among sows effect their progeny's gut microbiome and immune system development (Carney-Hinkle et al., 2009). A mammals GIT development and microbiome is determined through genetics, but also through diet and environment.

In conclusion, the age, bacteria present, and diet are three main factors that affect the microbiome of the host and thus their overall health (Buddington and Sangild, 2011). Through coevolution of the host's GIT and its current bacteria, there has been a commensal relationship that is developed that is not only species specific, but also

individual specific (Buddington and Sangild, 2011). Bacteria present within the gut have life long lasting immune and growth properties.

Effects of Dietary Habits

Dietary habits have a great influence on the composition of the gut microbiota. Factors including plant-type, farming practices, substrates, food composition and processes, and environment all cause a specific environment in which certain microbes can survive. While diet is known to influence gut maturation, it also helps to establish the gut microbiome (Buddington and Sangild, 2011). The gut microbiome is a crucial part of the body and has many immune attributes; therefore, in knowing the effect diet can have on the microbiome, altering diet can increase health and growth performance of the pig.

It has been known that obese subjects harbor greater amounts of bacteria that harvest energy. In a study conducted by Ridaura et al. (2013), where twins discordant for obesity were observed and their fecal matter were fed to mice there was a correlation in phenotype and bacteria. Female twins from 21-32 years in age included in the study in which their fecal matter was immediately frozen after being produced and then later given to germ-free 8-9-week-old male mice. When mice fed the obese feces and mice fed the lean feces were cohoused, there were no phenotypic characteristics of obesity. Furthermore, the obese fecal fed mice's microbiota was transformed to be more similar to that of a lean mouse. In the obese and lean mice that were cohoused there was an increased amount of Bacteroidetes. Interestingly, studies in which a low presence of Bacteroidetes was observed is often seen in obese subjects. Specifically, this study has shown how diet, environment and microbiota can greatly affect their host and their body-type. However, Bacteroides are genus within the phylum Bacteroidetes can degrade

complex polysaccharides and breakdown proteins to form the mucus above epithelial cells (Levast et al., 2013). Having beneficial bacteria species in gut microbiota prevent the multiplication of pathogens by simple competition for available nutrients and having the correct ratio of each bacteria greatly alters the health of the pig.

In a study conducted by Pedersen et al. (2013), genetics of pigs were seen to not have as much of an effect on microbiome as expected. In this study, 6 cloned pigs were used to study the effects of diet on the gut microbiota which decreases the variation associated with, for example, genetics and litter of origin. The non-cloned pigs and the cloned pigs were fed a high caloric/fat diet for 136 days to assess difference among the bacteria present in the gut. It was observed that there was difference in abundance of Bacteroidetes and Firmicutes based on weight. Body weight was positively correlated with the abundance in Firmicutes and negatively correlated with the abundance of Bacteroidetes. These results agree with other research studies and what has been previously stated about the importance of the ratio of Bacteroidetes and Firmicutes. This study was like the previous study by Ridaura et al. (2013), in which obese specimens had altered microbiome. While genetics will affect microbiome it is shown to had less of an effect than diet and environment due to the clones not showing any more similarity in microbiome than siblings in the trial (Pedersen et al., 2013). The microbiome of an animal is easily impacted and can have lasting effects on its health.

There are many different foods that can also affect the gut microbiome. Whole grain products are high in dietary fiber. Due to humans and pigs, having decreased ability to digest fiber when ingested, the microbiota is what help metabolize fiber and while fiber helps aid the growth of different bacterial populations (Graf et al., 2015). High

concentration of short-chain fatty acids and proteins promote growth of bacteria in the small intestine, whereas, in the large intestine, most of the available nutrients for bacteria are derived from indigestible carbohydrates and resistant starch as well as undigested protein in the diet (Sonnenburg et al., 2016; Fan et al., 2017). Furthermore fiber is a main energy source for gut microbiota and with altering levels in gestation diets it can help increase feed intake (Barnett et al, 2017) and it is believed to have significant effects on the composition and diversity of microbiota (De Filippo et al., 2017).

The defense mechanisms of gut microbiota include competing with pathogens for mucosal binding sites and nutrients, the elimination of toxic substances and the ability to produce anti-microbial like substances (Cummings et al., 2004). The microbiome stimulates local immune cell proliferation, thus playing a large part in the innate and adaptive immune system. Interestingly, there are major changes in the sow microbiome during gestation and lactation.

Due to the importance of the gut microbiota, choosing a diet for animals that enhances beneficial bacteria is ideal. Diet of the sow not only affects her, but also her offspring through skin contact and nursing. Altering the diet of a sow with various feedstuffs and maintaining a healthy weight promotes beneficial diverse bacteria to aid in digestion and immune response that can be passed onto offspring.

Neonate

The neonatal time is a critical period for intestinal maturation due to the GIT adapting to environmental factors, nutrition, and the gut microbiota (Walker et al., 2013). There are many beneficial effects that come from mammalian milk due to bioactive components that are present in milk. Directly after birth, a newborn’s GIT must establish

and maintain the fine line of recognition and exclusion of beneficial and harmful bacteria. Due to a fetus being in a sterile unit until birth and almost eating immediately after birth, it is important for the GIT to adapt to its new diet (Buddington and Sangild, 2011).

Within 12 h of being born, the GIT of a newborn goes from being sterile to being inhabited with bacteria at amounts like adults (Mackie et al., 1999). Infants that are delivered vaginally are colonized with bacteria that is originally from the mother's GIT, whereas infants delivered through caesarian are not exposed to these things and instead their initial bacteria come from their new environment. Infants however, whether they are delivered vaginally or via c-section, continue to acquire bacteria through their mothers' skin and breast milk (Hurre et al., 2008).

Breastmilk helps with the GIT, immune, and cognitive development during the rapid growth period of the neonate (Donovan et al., 2012). As found by Saulnier (2013), as the body grows, microbes and their metabolites play a key role in mediators of the gut-brain axis, thus showing microbes have functions far beyond just the GIT.

Microbial population of the fetus depends on maternal nutrition and maternal environment, as these both alter her microbial population (Macpherson et al., 2017). Bacteria that originates from the intestinal microorganisms of the mother influence the offspring through the placenta during fetal development, and later through maternal milk during the lactation phase (Macpherson et al., 2017). It has been shown that giving a sow a probiotic during gestation also greatly affects the piglet's microbiome (Starke et al., 2013). During the process of suckling, piglets are not only getting the value of nutrients needed for survival but also the immune defense needed as they begin to grow and encounter numerous pathogens. The effect of sow diet on piglet microbiome are largely

unexplored, but as more studies are being conducted more information on how to alter a neonate's microbiome in the first part of its life (in-utero and nursing) are being discovered.

Formula vs Maternal Milk

Due to greater number of piglets resulting in greater competition for nursing, pork producers are beginning to use milk replacer as a means to reduce mortality and morbidity among the litter (De Vos et al., 2014). Understanding the differences in formula and dam's milk can help to better produce a formula to meet the needs of a neonate and further explain the importance of a piglet being able to suckle from the sow. Piglets among larger litters are at a disadvantage if the sow is unable to produce enough milk to adequately supply the entire litter.

Polyamines, which contain two or more primary amino groups, have been shown to be involved in anti-inflammatory roles and intestinal epithelial barrier function. Furthermore, breast milk has a 10 times greater concentration of polyamines when compared to that of manufactured replacement formula. Continuing, polyamines appear to morph the gut microbiota composition in a positive way (Yeruva et al., 2016). Infants who are breastfed are reported to have less incidences of disease when compared to formula fed infants due to the increase amounts of lactic acid producing bacteria in nursing infants (Penders et al., 2006). Furthermore, mammals who nurse from their dam have shown a reduced number of bacteria that adhere to the mucosa (Van Haver et al., 2009). Components of milk which have also been linked to affecting gut microbiota are immunoglobulins, oligosaccharides, lactose, lactoferrin, and lysozyme (Newburg, 2009).

In order to evaluate the effect of replacement formula on the GIT, Yeruva et al. (2016), used a piglet neonate model to simulate that of a human as many similarities exist between the species. Piglets were allowed to suckle for the first 48 hours of life facilitating the ingestion of colostrum before being randomly selected and put on formula for the next 20 days. At the end of the study, formula fed piglets had greater number of diarrhea cases, most likely due to the low lactic acid bacteria to *E. Coli* ratio that was observed.

In a study conducted by Berding et al. (2016), where 2-day old piglets were fed a piglet formula (Control) or a formula with polydextrose, galacto-oligosaccharides, and milk-fat globule membrane (Test), differences among the microbiome were observed. After being fed the Test or Control formula for 30 days a microbiome analyses were conducted in which it was found that Test piglets had greater amounts of Clostridium IV and Parabacteroides (Bacteroidetes) and less of Proteobacteria. Clostridium IV is the main butyrate producing group of bacteria present in the gut and are known to start harboring in the intestine of breast-fed infants in the first month of their life (Nakano et al, 2012) Furthermore, Clostridium IV is correlated with maintaining the gut function of the infant (Nakano et al, 2012). Parabacteroides are also beneficial microbes that benefit the gut by eliminating potential pathogens from forming in the gut (Nakano et al, 2012). Furthermore, the lower amounts of Proteobacteria found in the test subjects, specifically, Escherichia/Shigella also have health benefits to the piglets. These microbes are correlated with acting as opportunistic pathogens in immunocompromised subjects – such as the newborn piglets. Also, piglets suffer from scours quite often and Escherichia coli and Shigella are associated with infantile diarrhea (Lanata et al., 2013).

A newborn piglet's GIT and microbial population is easily influenced due to its sterile gut; therefore, creating an ideal gut microbiota with beneficial bacteria right away can carry many health benefits through shaping the microbiota population as the piglet grows (Guarner and Malagelada, 2003). Diarrhea is the leading cause of neonatal and young piglet death; furthermore, it has been concluded that microbial pathogens, genetics, and nutrition are main players in this disease. Specifically, the gut microbiota is a factor in the cause of piglet diarrhea (Herman-bank et al., 2015). When a piglet is still nursing, it has an unstable microbial environment and weak immune response, making them more susceptible to illnesses (Bauer et al., 2006). Frese et al. (2015), found that the fecal biome of piglets from birth to weaning was significantly affected by the dietary glycans in the milk. In a study where Yang et al. (2015), collected 10 fecal samples from healthy pigs and 10 from piglets with diarrhea, it was observed that piglets with diarrhea had decreased *Firmicutes* and increased *Bacteroidetes.* The work by Yang et al. demonstrates how easily the microbiome can be changed and how the gut adapts to what is going on in the hosts body.

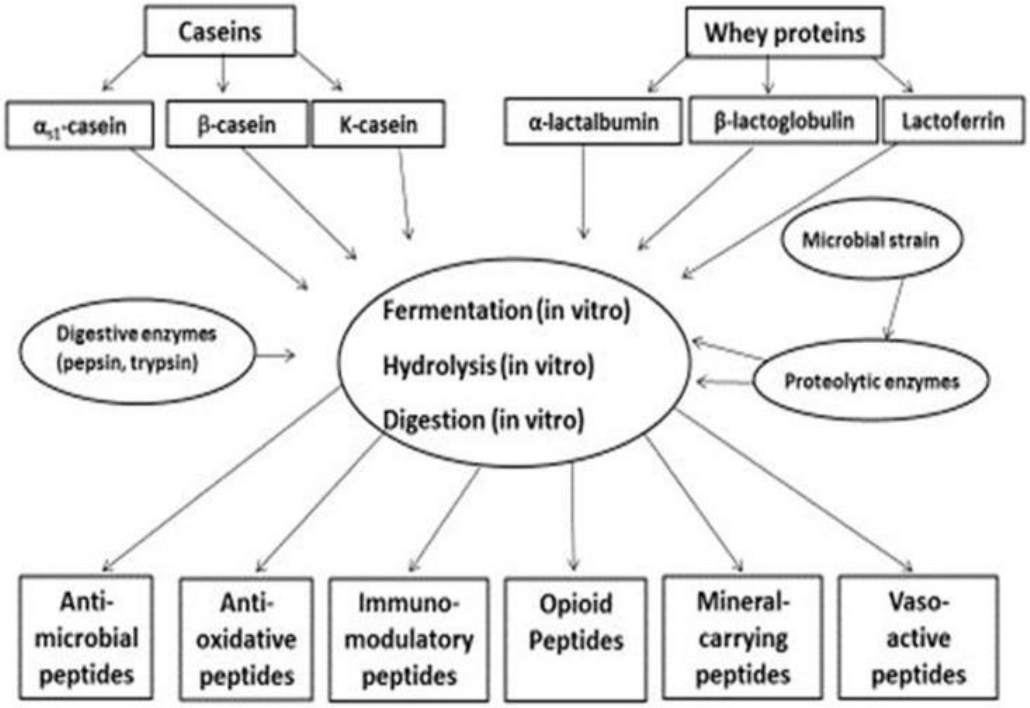
In a study conducted by Poulsen et al. (2016), where newly weaned piglets were fed bovine colostrum, milk replacer or sow's milk it was concluded that microbial colonization of the stomach, small intestine, and colon varied based on diet. The piglets fed milk replacer had a higher abundance of Enterobacteriaceae which is commonly associated with post-weaning diarrhea and was also supported observations made by Poulsen et al. (2016).

A mother's milk is the ideal nutrition for its offspring as it provides many bioactive ingredients to enhance the suckling pig's health and quality. Feeding the dam, a specific

diet for her to produce milk to best alter the infant's microbiome allows for piglets to stay healthier.

Milk Peptides

In 2001, peptidomics was introduced and described as the comprehensive characterization of peptides within a biological sample (Schulz et al., 2001). Today, peptidomics is being used more frequently for the characterization of nutritionally relevant bioactive peptides in food, specifically milk (Lahrichi et al., 2013). Bioactive peptides are specific protein fragments that have a positive influence on the physiological and metabolic functions of the body (Kitts and Weiler, 2003) and can be utilized by the body through ingestion and digestion of conventional foods, dietary supplements, and medical foods (Park and Nam 2015). There are numerous bioactive molecules within milk, in which among those molecules are bioactive peptides. These bioactive peptides possess the ability to affect, for example, the functionality of antimicrobial properties, antioxidative properties, and mineral-carrying activities (Park and Nam, 2015). Functional peptides in milk are usually derived from the proteins, casein and whey (Nielsen et al., 2017). Peptides derived from whey proteins are more quickly absorbed than Casein derived peptides (Brandelli et al., 2015). Typically, the bioactive proteins in milk are latent or incomplete in their original protein and become active due to proteolytic digestion (Park and Nam, 2015). As seen in the figure below (Korhonen and Pihlanto, 2007), bioactive peptides from milk are released in 3 ways: hydrolysis by digestive enzymes, hydrolysis of proteins by proteolytic microorganisms, and the action of proteolytic enzymes derived from microorganisms (Korhonen and Pihlanto, 2007).



Bioactive peptides have been identified within the amino acid sequences of native milk proteins (Park and Nam, 2015). Milk-born bioactive proteins, including whey and casein, break down into peptides that impact health and metabolism of the infant. Furthermore, bioactive milk peptides fall into four descriptive categories: (1) gastrointestinal development, activity, and function; (2) immunological development and function; (3) infant development; and (4) microbial activity, including antibiotic and probiotic action (Korhonen and Pihlanto, 2007).

There are several factors that potentially influence the bioavailability of milk peptides. Numerous studies have indicated that gastrointestinal transit rate plays a key role in determining the use of bioactive peptides in the body (Ledoux et al., 1999). Also, protein dissimilarity among different milk samples (i.e., cow vs human) may bring forth different bioactive responses within the body in regard to a neonate consuming breastmilk or formula. After the ingestion of milk, peptide size, weight, and properties

determine the major route of transport directly relating to the peptide's bioavailability (Shimizu et al., 1997). Proteolytic enzymes from different lactic acid bacteria can produce a variety of different peptides depending upon the cleavage site (Giacometti and Buretic-Tomljanovic, 2017). Milk peptides have many beneficial effects on a suckling neonate, but the diet of the sow can greatly affect this through diet and environment in which microbiome is altered.

Conclusion to the Effects of the Maternal Diet on Progeny Health & Growth

Pig production profitability is related to the animals' efficiency. Thus, a sow having greater reproductive performance such as litter size and litter uniformity increases their profitability. With larger litters resulting in a higher number of piglets weaned, getting uniform litters would lead to lower mortality rates and better post-weaning performance causing overall better profits for the producer. Through changes in the sow's diet, during gestation, milk composition will change and can result in a more nutrient defense milk while also altering the piglet's gut microbiome with possibly more beneficial bacteria. There is little research on the sow's gestation diet regarding piglet performance and more research needs to be on this topic.

Overall Conclusion

Being able to complete both a GTA and GRA have allowed me to better execute my animal research through sound hypotheses and increased critical thinking. While it is not typical for students in the Animal Science department to equally focus on both animal research and teaching research it has allowed to expand by knowledge in teaching and use that in connecting with professors and approaching my animal research from different angles. Through completing a GTA, I believe when I assisted or taught Animal Science

classes at UNL I was able to better explain topics and connect with students. Furthermore, through completing various teaching classes and working simultaneously in the lab, I was able to relate better to individuals in my lab when helping them learn new lab practices. While it is necessary to complete multiple animal experiments to graduate with a PhD and much scientific knowledge was gained, the teaching side of my PhD increased my job eligibility by having both the lab experience and teaching experience.

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Chapter 2. Impacts of Participation in Undergraduate Research on Students Majoring in Animal Science

Abstract: Undergraduates in science related majors that participate in research have shown potential benefits for both academics and careers. Undergraduate students participating or past participants of Animal Science research were asked to complete a survey that included a series of demographic questions, as well as, questions related to the perceived impacts of their participation in undergraduate research. On the survey, students were asked to rank statements on a 1 (not at all) to 5 (definitely) Likert scale to analyze the impacts of completing undergraduate research and the effect it has on one's knowledge and ability to use and apply what was taught. A total of 30 students completed the survey. Ten percent of students that participated in the survey were male and the other 90% were female. Students ages ranged from 18-23 with the most frequent categories being 20-21 (46%), seniors (33.3%), and Nebraska residents (78%). Students expressed that they heard about the undergraduate research opportunity from their professor (48%) or an outside source such as pre-veterinary club or class presentations (36%). Sixty percent of individuals indicated that pre-veterinary medicine was their current option within the Animal Science major. Only 4% indicated they did not plan to continue a higher degree postgraduation. Undergraduate students when directly asked who had a greater impact on their undergraduate research, graduate students ranked higher than the professor (43% vs 36%), while 21% of participants said neither had an impact on their research as an undergraduate. However, when asked to rate professors or graduate students on a Likert scale, both professors and graduate students were beneficial in their undergraduate research. Students felt many benefits from participating in undergraduate

research from educational opportunities, such as it helping in their current classwork to feeling more prepared for a career.

Introduction

Many students with a science, technology, engineering, or mathematics (STEM) major participate in undergraduate research within their home department. Participants are able to enhance their scientific learning skills and develop skills that will help their resume stand out, whether it be for their career or pursuit of higher education applications (Linn et al., 2015). Mervis (2001) found that 70% of students that planned to continue their education beyond a bachelor's degree had participated in some type of undergraduate research program. Lopatto (2004) found that 87% of the students who participated in the survey planned on continuing a higher education after participating in undergraduate research and only 4.5% of the respondents decided to discontinue their plans on further scientific education after doing undergraduate research.

For students in science-based majors, undergraduate research opportunities are beneficial to their GPA and influence students to pursue an advanced degree. According to a survey done by Russel et al. (2007), 68% of undergraduate students who participated in hands-on research had an increased desire to pursue careers within STEM fields, while only 8% expressed a decreased desire afterwards. Furthermore, mentors did not have an effect on an undergraduates perceived interest in receiving an advanced degree, however when asked what they would like to see improve in the future for other undergraduate research students, it was stated that they would want increased faculty guidance (Russel et al., 2007).

Many professional Animal Science focused conferences including the American Dairy Science Association, Equine Science Society, and American Society of Animal Sciences allow undergraduates to be recognized for their research and present their findings to the industry (Whittington, 2020). In addition to being recognized by professional societies, some undergraduates are also being recognized by their university, such as graduating with research distinction (Whittington, 2020).

University of Nebraska - Lincoln (UNL) provides a program called Undergraduate Creative Activities and Research Experience (UCARE). The UCARE program is funded by Pepsi Quasi Endowment and Union Bank and Trust. Through this program, undergraduates have access work one-on-one with faculty research advisors. Accepted students can participate in research involving anything from poems and music to science and math. Along with completing research and broadening their knowledge on a subject, undergraduates accepted for this program will also receive a stipend. The university acknowledges the importance of getting involved and rewarding those who are looking to enhance their education outside the classroom. Additional students are provided undergraduate research experiences within the Animal Science Department due to the amount of research conducted and need for additional personnel beyond graduate students. However, little work has been completed to evaluate the impacts that completing an undergraduate research project has on the student. Therefore, the primary goal of this survey is to determine the impacts of working in research as an undergraduate student and how it affects their future educational and career goals.

Materials and Methods

Respondents of the Survey

Animal Science students that have completed or are currently working in an undergraduate research program within the department were asked to complete a survey. The procedures of the survey were reviewed and approved by the University of Nebraska-Lincoln's Institutional Review Board (IRB). An assessment was provided to all Animal Science students through an Animal Science email listserv and only those who self-identified as having completed undergraduate research were asked to complete the survey. Students were sent two reminder emails two weeks apart. The evaluation was completely anonymous and no identifying information was collected. The evaluation included two parts: demographics and assessing student perception of the research experience (Appendix 1).

Description of Survey

An evaluation tool was developed to be completed by undergraduate research students in the Animal Science Department. The survey asked demographic information, including ethnicity and gender. The survey also asked students various questions including multiple choice, fill in the blank and 5-point Likert scale questions. On the Likert scale questions, students were asked to respond with a number (5 = Yes, very much, 4 = a little, 3 = Somewhat, 2 = not really, 1 = not at all) to a series of questions based on how much impact that statement had. Statement topics included the impact graduate students, professors, and research had on various aspects of their education and experience. Survey questions were designed to obtain feedback from students on how the research pertained to their future career goals, how each student interacted with graduate students and instructors, and how the research enhanced new and old skills, but not limited to their understanding of Animal Science and its relevance to their future.

Survey Analysis

Means and standard deviations of question responses were calculated for individual statements that were in the Likert scale type format. Statements in the Likert scale format were considered to have a significant impact if the average was great than 3. Percentages were calculated from questions that were multiple choice, thus not presented in a ranking type fashion (i.e. demographics).

Results and Discussion

Demographics

Thirty students completed the undergraduate research survey. It is unknown the total number of students completing undergraduate research at any given time in the Animal Science Department to estimate response rates. Similar to the trend in the Animal Science major, where 72% of students are female (Data index, 2008), a majority of survey respondents were also female (90%) and only 10% were male (Table 1a). National trends also show an increase in women within Animal Science departments (Esbenshade, 2007). Furthermore, 100% of the respondents were white and American citizens and 78% were Nebraska residents (Table 1a). The above demographics are similar to that of the Animal Science department at UNL in which, 70% are female and 92% of the undergraduate students are white. Also, in the College of Agricultural Sciences and Natural Resources, 75.8% of the students are Nebraska residents (Data index, 2008). Recruitment efforts are underway to increase the number of non-resident students and increase diversity within the department. Similar efforts are needed within the undergraduate research program to improve the experience of students.

Animal Science departments have seen changing demographics. Today, a greater number of students are identifying as being from an urban or city population as opposed to students raised with an agricultural background (Buchanan, 2008). This current study showed over 60% of students reported coming from a city of greater than 5,000 people (Table 1a). With a growing number of students coming from an urban background, there needs to be additional opportunities for students to gain experience with agricultural animals (Karcher and Trottier, 2014).

Of the students that completed the survey, 13.3% were freshmen, 26.7% were sophomore, 26.7% were junior, and 33.3% were seniors, resulting in over 59.0% upperclassmen (Table 1b). While studies have shown the benefits of research experience early, many undergraduates are not aware of or do not take advantage of these opportunities until later in college causing a possible reduction in STEM students (Russel et al, 2007). Students who participate in research in the first 2 years of college are more likely to continue in STEM majors (Nagda et al., 1998). Also, due to introductory courses possibly impacting a student's opinion on a topic if students perceive it as being boring or too much busy work, or if classes are too hard and professors are not able to relate to them (Seymour and Hewitt, 2004) can cause students to change majors, but getting students in undergraduate research can help deter students from changing their STEM majors. Providing a hands-on experience that allows students to apply concepts learned in the classroom can increase student persistence in a STEM major.

Future Goals of Students

The majority of respondents (76%) planned to continue to an advanced degree, with only 4% not intending to continue, and 20% being unsure. Furthermore, 20% of

students had already been accepted into graduate school upon taking the survey and 24% of students in the survey were going to or planned on continuing their advanced degree at UNL (Table 2). These results are similar to what was found in Campbell and Skoog's (2008) research stating that participating in undergraduate research can be correlated to an increased retention in science and greater potential of attending graduate school compared to peers who did not work in research as undergraduates. Interestingly, students that participated in this survey stated undergraduate did not have a large effect on changing their minds about their academic path (mean = 2.6, SD = 1.58), this could possibly be because most students already planned on continuing their education as they were eager to begin their jobs as undergraduate researchers (mean = 4.0, SD = 0.99) (Table 4).

In respect to the future goals of the undergraduate research students, being able to work in a lab under Animal Science faculty enables a faculty-student relationship and can increase a student's chances of being accepted into graduate school, while also helping professors identify potential graduate students (Sterle and Bundy, 2018). Being an animal scientist entails formal training and adequate experience in order to use problem solving techniques when faced with animal production, care, and use issues. Ensuring that students are ready for life outside the classroom and prepared for a career in their chosen industry is the mission of universities and can be better achieved through undergraduate research and hands-on learning. Furthermore, according to the National Research Council there is a greater call for more problem-based learning in which students can put their knowledge to use (Araz and Sungur, 2007) such as through an undergraduate research program. According to Wei and Woodin (2011), students that participate in research

outside of the classroom or Animal Science clubs in a topic of interest develop a greater understanding for that subject. Professors of Animal Sciences should encourage undergraduates to participate in research outside the classroom to develop skills that will help them in their future endeavors because research has claimed that undergraduate research experience elicits greater preparation for future scientist (Graham et al, 2013).

UR work environment

Students responded to questions about the area in which they conducted their research (Table 3). Students in the Animal Science undergraduate research program completed between one and 20 hours of work a week with the majority of students (35.5%) working 6 to 10 hours a week. The average student had been working in a research program over the course of 3 to 5 semesters with 92.8% working 5 semesters or less. Students can participate in an array of research disciplines within the animal department from physiology to genetics to nutrition. The majority of students who were completing undergraduate research in an Animal Science laboratory were in the following areas: ruminant nutrition – beef cattle (27.7%), breeding and genetics (16.6%), physiology (11.1%), meat science (5.5%), ruminant nutrition – dairy cattle (5.5%) and other or labs selected by only 1 participant (33.3%).

Continuing, Allowing students with an Animal Science major to work in laboratories of their interest allows them gain greater knowledge in specific topics and find out what they are and are not interested in (Jones, 2019). As seen in the current study, students also reported that working in an animal science lab gave them a better perception (mean = 3.6, SD = 1.38) and respect of animal research (mean = 4.2, SD = 1.04) when compared to their previous knowledge as well as their interest in working in

research (mean = 3.4, SD = 1.49) (Table 4). Through working in research, students are experiencing discovery and innovation in their major and feeling motivated as they begin producing data results (Gentile et al., 2017). Furthermore,

Self-Evaluation

Students were asked to evaluate the specific skills earned from completing undergraduate research (Table 4). Students indicated that undergraduate research had a moderate benefit to them in the classroom (mean = 3.0, SD = 1.61). Students also indicated participation in undergraduate research changed their feelings in a positive way towards graduate school (mean = 3.2, SD = 1.59), and it prepared them for their future education goals (mean = 3.6, SD = 1.55). For those accepted into graduate school (20% of the respondents), it also allowed them to feel better prepared (mean = 3.2, SD = 1.81), students who have not been accepted to graduate school selected not applicable. Students agreed that the research was what they expected it would be (mean = 4.0, SD = 0.81) and they felt comfortable performing tasks on their own (mean = 4.5, SD = 0.52). According to Graham et al. (2013), research experience is a useful learning tool engage students and encourages professional identification.

Many benefits come from working in a science lab. Jones (2019) found that in study conducted on 556 Animal Science undergraduates, undergraduate research experience improved the critical thinking ability of Animal Science students. This current study found that working in a lab benefited them scientifically in many ways including, critical thinking (mean = 4.1, SD = 0.86), ability to support a hypothesis (mean = 4.1, SD = 0.76), application of the scientific method (mean = 4.3, SD = 0.62), communication skills (mean = 3.7, SD = 1.12) and listening skills (mean = 3.6, SD = 1.07).

Interesting, students who responded to this survey stated that undergraduate research did not help improve their computer skills, including programs: Microsoft word (mean = 2, SD = 1.75), PowerPoint (mean = 2.1, SD = 1.7), Excel (mean = 2.7, SD = 1.84) and Statistical programs (mean = 1.6, SD = 1.48). This could be because students were mostly working in the lab following protocols and working with live animals were these types of programs are not needed.

The undergraduate research at UNL strives to produce successful researchers by developing a specific program to target these students known as UCARE, posting job openings on the undergraduate bulletin, and making job announcements in classes and clubs. If universities develop skill-building seminars on topics such as creating research posters and how to properly present one's data, the value of the experience as an undergraduate research can help set a student up for its future (Council on Graduate Research, 2020).

Mentorship from Graduate Students and Faculty

Undergraduate students reported a positive relationship with faculty (mean = 4.6, SD = 0.87) and graduate students (mean = 4.2, SD = 1.42; Table 5). Students reported that they felt faculty (mean = 4.3, SD = 1.18) and graduate students (mean= 3.7, SD = 1.36) were eager to teach them. Students noted they received adequate training in working with research animals (mean = 4.2, SD = 1.48) and lab equipment (mean = 3.7, SD = 1.78) (Table 5). Additionally, students agreed that if they were unsure, they felt comfortable asking questions (mean = 4.4, SD = 0.78). The reason students felt comfortable may be because they felt the attitudes of graduate students they worked with and faculty they worked were near excellent (mean = 4.4, SD = 0.87; 4.3, SD = 0.62,

respectively). In a statistical analysis of over 5,000 undergraduate research students, a positive experience in working with faculty and graduate students overseeing their work was noted (Lopatto, 2010). Due to the positive experience from undergraduates in the current study, over 75% of the students would “Yes, very much so” recommend other undergraduates to work in the same laboratory and 70% would “Yes, very much so” recommend undergraduate research at UNL as they felt it was a good use of their time (mean = 4.3, SD = 0.99). Similarly, students in a 2007 survey reported that the development of relationships within their laboratory group was their number one benefit (Hunter et al., 2007). Furthermore, it has been reported that working in a laboratory group helped the students feel a sense of belonging (Hunter et al., 2007). Professors and graduate students helping undergraduate researchers should work to form connections among experiences with experimental design, data collection, interpretation of findings, and scientific communication, thus preparing students to understand science concepts and practices necessary for higher education and a future career. Mentors should support students to develop professionalism and emotional strength in a field that can have many setbacks (Schwartz, 2012). Additionally, mentors provide professional socialization and emotional support to students allowing for greater confidence in the student and are less likely to push students to change their major (Thiry et al., 2011). Additionally, students that responded to this survey agreed that faculty and graduate students encouraged and supported their research by allowing them to seek advice from graduate students (mean = 3.4, SD = 1.45) and faculty (mean = 4.0, SD = 1.00). Whether the mentor of an undergraduate research student is faculty or a graduate student, they play a key role in experience and preparation for future in endeavors. Among all the hours spent in

research, an undergraduate is usually overseen by an abundance of people. Mentoring is typically shared among faculty, postdoctoral researchers, and graduate students. The abundance of mentors allows the undergraduate to have many one on one learning opportunities. However, studies show undergraduates interact most often with graduate students and postdocs, and less with professors (Thiry and Laursen, 2011). This is similar to the results of our study in which more students indicated that graduate students had a greater positive impact on their undergraduate research experience compared to that of professors. Interestingly, Feldman et al, (2013) stated undergraduates that worked primarily with graduate and post-doctoral researchers tended to focus on technical aspects of the projects, yet when they worked with the professor, the professor tended to help students build on skill of knowledge, reasoning, and problem-solving.

Undergraduate research enhances student learning through mentoring relationships with faculty members, increases retention of the academic program and graduation rates, and develops an understanding for research methodology (Council on Undergraduate Research, 2020). Undergraduate research heightens the skills of students by building relationships with faculty, developing better time management, and learning to think outside the box (UCARE). In a cross-institutional study on the benefits of undergraduate research it was found that it promotes gains in skills, self-confidence, pathways to science careers, and active learning (Lopatto, 2004, 2007). Out of classroom interactions with faculty results in greater persistence in their major and greater academic integration (Milem and Berger, 1997).

Summary

The various students that responded to the survey were a good representation of the demographics of the Animal Science department at UNL allowing for an accurate portrayal of the department. The study concluded that involvement in undergraduate research is one way to help build the skills needed for a scientist. An education in Animal Science provides undergraduate students with technical skills as well as theoretical knowledge in a diverse array of areas; however, through undergraduate research they can learn time management, critical thinking, and develop relationships with students and faculty. Acquiring skills in research, abled students to use what they have learned in other aspects of their life (Robinson and Mulvaney, 2018) and was also shown in the current study. Students stated that they have been able to use their newly attained knowledge from undergraduate research in their classes and in obtaining a higher degree. Undergraduate research has many benefits and should be a focus in the Animal Science department to help set students up for success.

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Table 1a. Respondents Hometown population. Percentage of respondents who were raised in town/city population of <1000 people to >10,000 people. (N = 30)

People	Percentage
<1000 people	14.2%
1000-5000 people	25.0%
5001-10,000 people	17.8%
>10000 people	42.8%

Table 1b. Percentage of responding Animal Science students who completed undergraduate research reported gender, year in school, age, and ethnicity. (N = 30)

Item	Percentage
Gender	
Male	10.0%
Female	90.0%
Level in College	
Freshmen	13.3%
Sophomore	26.7%
Junior	26.7%
Senior	33.3%
Ethnicity	
White	100.0%
Nebraska Resident	
Yes	78%

No	22%
U.S. Citizen	
Yes	100%

Table 2. Future plans of undergraduate Animal Science students who participated in research. (N = 30)

Item	Percentage
Want to Continue to an Advanced degree	
Yes	76.0%
No	4.0%
Unsure	20.0%
Accepted to Graduate School	
Yes	20.0%
No	64.0%
No Applicable	16.0%
Attending UNL for advanced degree	
Yes	24.0%
No	40.0%
Unsure	36.0%
Attending graduate or professional school the academic year	
Yes	56.0%
No	12.0%
Unsure	32.0%

Table 3. Hours worked, length of research experience, and discipline of research for undergraduate Animal Science student researchers. (N = 30)

Item	Percentage
Typical hours worked per week	
1-5	21.4%
6-10	35.7%
11-15	28.5%
16-20	7.1%
>20	7.1%
Number of semesters in undergraduate research	
1-2	42.8%
3-5	50%
6-8	7.2%
9+	0.0%
Research Discipline	
Ruminant Nutrition – Beef	27.7%
Breeding and Genetics	16.6%
Physiology	11.1%
Ruminant Nutrition – Dairy	5.5%
Meat Science	5.5%
Non-Ruminant Nutrition - Swine	0.0%
Non-Ruminant Nutrition – Poultry	0.0%
Other	33.3%

Table 4. Perceived impacts of undergraduate research on undergraduate Animal Science students (N = 30)

Item[1]	**Mean**	**SD**
Has helped me in classes	3.0	1.62
Has changed my feelings in a positive way about grad school	3.2	1.59
Has prepared me for graduate school[2]	3.2	1.81
Relates to my future education goals	3.6	1.55
The research I participated in is what I expected it to be	4.0	0.82
I applied concepts I learned in classes, while working on research	3.5	0.87
Felt Comfortable performing tasks on my own	4.5	0.52
Improved my critical thinking	4.1	0.86
Improved ability to support a hypothesis	4.1	0.76
Application of the scientific method	4.3	0.62
Communication skills	3.7	1.12
Perception of animal research	3.6	1.38
Interest in working in animal research post-graduation	3.4	1.38
Eager to begin work as an undergraduate researcher	4.0	0.99
Undergraduate research changed my academic path	2.6	1.58
Improved my skills in Microsoft Word	2	1.75
Improved my skills in PowerPoint	2.1	1.7
Improved my skill in Excel	2.7	1.84
Improved my skills in statistical programs	1.6	1.48

[1]Ranked on a scale of 1-5: 5 = Yes, very much, 4 = a little, 3 = Somewhat, 2 = not really, 1 = not at all

[2]N = 14 (only completed by students planning to attend graduate school)

Table 5. Perceived impacts of training and faculty and graduate student mentorship on undergraduate research experiences of Animal Science majors (N = 30)

Item[1]	**Mean**	**SD**
I received adequate training before working with research animals	4.2	1.48
I received adequate training before working in the lab with chemicals	3.7	1.79
Positive relationship with the faculty during undergraduate research	4.6	0.87
Positive relationship with the graduate during undergraduate research	4.2	1.42
Adequate availability of the faculty within the lab you worked in for when you had questions	4.6	0.63
Adequate availability of the graduate students within the lab you worked in for when you had questions	4.3	1.38
Seek advice from the faculty members of this lab for future career plans	4.0	1.00
Seek advice from the graduate students of this lab for future academic plans	3.4	1.45
Graduate students within the lab group were eager to teach me	3.7	1.36
Faculty within the lab group were eager to teach me	4.3	1.18
I felt comfortable asking questions	4.4	0.78
Recommend this lab group to other students	4.6	0.63
Recommend University of Nebraska - Lincoln to incoming freshmen	4.3	1.19
The attitudes of the students worked with	4.4	0.87
The attitudes of the faculty worked with	4.3	0.62

Faculty worked with was respectful to me	4.6	0.62
Graduate students were respectful to me	4.1	1.34
Working the lab was good use of my time	4.3	0.99

[1]Ranked on a scale of 1-5: 5 = Yes, very much, 4 = a little, 3 = Somewhat, 2 = not really, 1 = not at all

Chapter 3. Assessment of Undergraduate Student Learning in an Animal Science Major

Abstract: With changing demographics of undergraduate students in the Animal Science major it is important to evaluate the curriculum and student learning. There has been a large shift from students of a rural agriculture background to more students being from a non-agricultural background. Furthermore, the increase in female prevalence has also been a change in the Animal Science department. Thus, with the many changes in students, the objective of this study was to assess perceived and actual knowledge gained by students in the Animal Science major in order to address any areas needing improvement within the department and to aid in students' success. Results of this evaluation have been compiled from years 2015-2017 with 253 students responding. As expected, there were no ($P > 0.05$) differences over time in the categories of: understanding, skills, attitudes, integration and knowledge-based questions. However, results indicate that students perceived enrolling in the Animal Science major improved the areas of understanding, skills, attitudes, integration of learning and knowledge. However, an additional focus on student understanding of basic sciences and communication skills could be beneficial. Lastly, in evaluating student's recollection of information based on core Animal Science concepts, overall student scores were higher ($P = 0.0002$) in the areas of nutrition and meat science. Student scores were lower in advanced subject areas. This may be due to either difficulty of concepts or students having not completed the courses yet. Overall, students rated the Animal Science major as meeting their expectations in both knowledge and skill development. Additional focus

on the importance of critical thinking, communication skills and application of concepts may improve student satisfaction.

Introduction

An education in Animal Science provides undergraduate students with technical skills as well as theoretical knowledge in a diverse array of areas including animal behavior, management, genetics, nutrition, physiology, and reproduction. In addition to basic Animal Science knowledge, there are specific skills students are projected to use throughout their future career (Forsberg et al., 2003). Being an animal scientist entails formal training and adequate experience in order to use problem solving techniques when faced with animal production, care, and use. Ensuring that students are ready for life outside the classroom and prepared for a career in their chosen industry is the mission of universities. Through assessing the needs of their students, a university can better prepare its students (Robinson and Mulvaney, 2018). For example, it has been found that basic computer skills and the ability to interpret data are some of the most sought-after technical skills in the Animal Science industry (Robinson and Mulvaney, 2018). Due to the rapid changes in technology, universities are always being called upon to update their programs to keep their students competitive in the workforce after graduation (Robinson and Mulvaney, 2018). Furthermore, according to the National Research Council there is a greater call for more problem-based learning in which students can put their knowledge to use (Araz and Sungur, 2007). Teachers of sciences are beginning to incorporate a broader spectrum of examples in classes and being encouraged to develop a curriculum that allows students to engage and participate in independent research and scholarship competitions. Students should be able to possess a variety of skills at the time of

graduation. The goal for an undergraduate is to leave college with the knowledge of a new subject and the development of a talent in which these can be used to create success outside the walls of the university (Jones and Lerner, 2019).

The Animal Science Department at the University of Nebraska – Lincoln facilitates a diverse learning environment in both technical and theoretical based areas. Additionally, the demographics of enrollment in the Animal Science department has continued to change. Currently, there is an increasing proportion of female students enrolled. From 2010 to 2018, the proportion of male students has decreased from 38% to 29% and the number of female students increased from 62% to 71% (UNL, 2020). These results are similar to those of the University of Michigan, which reported 73% of students majoring in Animal Science were female in 2014. Nationals trends show an increase in women within the Animal Science department have also been reported. Gender is not the only big change Animal Science departments have seen among changing demographics. Today, a greater number of students are identifying as being from an urban or city population as opposed to students raised with an agricultural background. In a survey conducted by Iowa State University, 42% of students stated they were from a rural/farm town whereas 58% stated they were from a city (Sterle and Tyler, 2016).

Responding and adapting to changing student demographics, combined with facilitating the attainment of skills desired by future employers is vital for ensuring that students thrive in an Animal Science-based education environment and post-graduation success. Therefore, the objective of the study was to evaluate student learning in an

Animal Science program and to assess the development of skills required to be successful in graduate school or a career in the Animal Science industry.

Materials and Methods

Respondents of the Survey

A survey was conducted to meet the objectives of this study. Data was collected from undergraduate students who were enrolled as animal science majors in the fall of 2015.The was approved the university of Nebraska-Lincoln IRB and participants provided consent by completion of the survey Animal Science students in their last year of their undergraduate program were required to enroll in a senior seminar course. An undergraduate senior exit evaluation was administered to students in this course to assess student outcomes (perceived and actual knowledge).

An assessment was provided to students during the last two weeks of the Animal Science senior seminar course. The senior seminar is taught each semester (fall and spring) to undergraduate seniors and required for graduation for Animal Science majors. Data was collected both spring and fall semesters from years 2015-2017 for this study at the University of Nebraska-Lincoln. During this time, 283 students were asked to complete the evaluation, where one part was assessing knowledge, one part was collecting demographics and the other part was assessing subjective thoughts on student satisfaction. The survey had an 89.4% completion rate. Table 1 shows the number of students each year that completed the evaluation. The instructor of the seminar was the Animal Science department chair. Respondents were also asked to identify their current choice of major at the end of this course.

Description of Survey

The Animal Science assessment was administered to seniors within this major to collect data on student satisfaction, student perceived knowledge, and actual knowledge gained, and to provide data to help the Animal Science Department to respond and adapt to changing student demographics (Appendix 2).

The assessment tool included multiple sections. Information was collected on student reported grade point average (GPA) and major. A section consisted of survey questions requiring 5-point Likert-type scale response to allow individuals to express how much they agree with a particular statement (e.g., a response of 1, indicates "not at all", whereas a response of 5, indicates "a great deal"). Students were asked to respond to statements regarding how they felt their understanding, skills, attitude, and integration of learning developed during matriculation in the Animal Science major.

In the final section, students were asked to respond to twenty knowledge-based questions from the core Animal Science courses. These multiple choice questions were in the topics of physiology, meat science, nutrition, and genetics. Questions were submitted by instructors of the core courses in these areas. Questions were not validated but were concepts the instructors felt students should learn and retain from their courses. Students' answers were then analyzed based on which topic the question fell into and trends based on year were assessed. The survey and exam questions remained consistent for the three-year period.

Procedure

The instructor of the senior seminar class distributed the survey instrument during one of the last two class sessions. Students were asked to complete it before the end of

the period. The evaluation was completely anonymous and no identifying information was collected.

On the 5-point scale, ranging from 1 (“not at all”) to 5 (“a great deal’), students rated: their understanding, skills, attitude, and integration of learning pertaining to their time spent in the Animal Science department. Answers among all 6 semesters were combined and assessed by individual question

The knowledge-based part of the survey was scored on a correct or incorrect basis and overall scores were analyzed in JMP 12 (Jmp, 2019) to test for statistical differences among topics. Score per subject area (e.g., nutrition, genetics) were also analyzed separately.

Statistical analysis

Data was analyzed in JMP 12 (Jmp, 2019). For the objective part of the test, data was analyzed using LSMeans differences in a Tukey HSD report. Data are presented as standard means and $P < 0.05$ was considered significant. Data was analyzed across all semesters and then analyzed as a lump sum to increase power.

Results and Discussion

The overall response rate of the survey was 89.4% ranging from the lowest of 80.0% in spring 2016 to 95.9% in fall 2016 (Table 1).

Major:

Students were asked whether or not they were currently an Animal Science major. Over 91% of respondents were majoring in Animal Science (Table 1). Of the 8.5% students who were not Animal Science majors, 91.6% still had majors within the College of Agriculture and Natural Resources and 50% of those students were majoring

in Agribusiness. Animal Science Senior Seminar is a required class for all Animal Science majors during the last year of their program. The course does not meet a degree requirement for other majors besides Animal Science. Students from these other majors may have been taking the senior seminar course to fulfill a portion of an Animal Science minor. While students minoring in Animal Science will not have taken all the core classes as those who are majoring in it, they should still benefit from the select Animal Science classes they take and impact of those courses were still considered in analyzing surveys.

GPA:

Students self-reported their GPA. There was no significant difference among GPA based on year and trends are similar throughout (Table 2). A majority (62.3%) of the students reported a 3.01 GPA or above across all semesters. Among all six semesters, there were fewer students averaging a 2.0 or below GPA compared to students with a GPA of 3.0 or higher ($P < 0.05$). This is important to note as a higher GPA can be correlated with efficient studying practices and higher understanding (Plant et al., 2005). Furthermore, students with less than a 2.0 GPA are placed on academic probation. It has been shown that a higher GPA has a positive relationship with earnings and job satisfaction post-graduation (Vermeulen and Schmidt, 2008). It has been noted that alumni with high grades during their studies were later more successful when compared to those with a lower GPA (Vermeulen and Schmidt, 2008).

Undergraduate GPA can play an important role in the success of the student whether it be in graduate school or in their career (U.S. Department of Education, 2008). Employers have been known to put emphasis on a student's GPA when reviewing a

resume and considering them for hire (Nelson, 2008). One study showed that resumes with high GPAs were significantly more often selected for job interviews than identical resumes with lower GPAs (Thoms et al., 1999). According to a study done by Boston University School of Medicine, students that had a higher GPA during their undergraduate career had greater success in graduate school (Park et al., 2018). Furthermore, a student's GPA was one of the greatest indicators for predicting students' success in their career post-graduation (Park et al., 2018). GPA shows a positive correlation with graduate school and career success (Nelson, 2008). The fact that the majority of students have a GPA > 3.0 may indicate that students more successful in Animal Science coursework were more likely to persist to a degree.

Effects of major on understanding, skills, and attitude

Because there was no significant difference between semesters, data from all the semesters was combined to analyze overall effects of the curriculum. The total number of responses were tallied for each individual question based on students' response for a quantitative way to analyze effects of major on perceived knowledge of better understanding certain topics, establishing specific skills, and their feelings toward their undergraduate program in Animal Science (Table 3-Table 6). In addressing a student's perspective, it has been found that better understanding can result in increased learning and critical thinking for the student (Swart, 2017).

Over half the students across all semesters indicated the Animal Science curriculum increased their understanding of how biology and chemistry of the life sciences apply to Animal Science principles by "a lot" or "a great deal" (n=178, 70%). Interestingly as shown in Table 3, the statement, "How ideas we explore in my biology

and chemistry classes relate to my Animal Science classes" had the lowest response of less than 52% answering "a lot" or "a great deal" among all six semesters (n=137, 54%). Students noted a higher rate of understanding when applying biology and chemistry to life sciences and their Animal Science courses than in their biology and chemistry courses alone. The American Association for the Advancement of Sciences calls for biology teachers to update teaching methods to better accommodate students of the 21st century (AAAS, 2011, National Research Council, 2009). In order to better accommodate, teachers would have more hands on learning and undergraduate research available and connecting the science taught in class to real world issues (AAAS, 2011). Animal Science curriculum should be updated so that students better understand the importance of general biology and chemistry as a foundation for Animal Science.

Students may be able to apply biology and chemistry in their Animal Science courses better than in their biology and chemistry courses due to increased interest and greater opportunities to use what they have learned. A curriculum that emphasizes basic sciences, such as biology and chemistry coursework, and how it can later be applied in their major, may influence students to see the value in basic sciences. According to Wei and Woodin (2011), students that participate in research outside of the classroom or clubs in a topic of interest develop a greater understanding for that subject.

The highest percentage (n=236, 93%) of students noted "a great deal" of to "a lot" of improvement in the understanding of specific Animal Science disciplines and terms. Students also reported a high understanding of how Animal Science concepts can be applied to real world problems (n=219, 87%). These results agree with the idea that learning outcomes for students, especially in a science field, should support critical

thinking in which students are able to understand concepts they learned in their courses and know how to apply them to areas outside of the classroom (Holmes et al., 2015). According to the National Research Council, it is a goal to support students in thinking critically within agricultural sciences, but not all classes present students the opportunity to do so (NRC, 2009). Many Animal Science programs within agricultural departments have livestock competitions, meat judging competitions and undergraduate research programs that allow students to put their knowledge to use outside of the classroom.

When looking at the effects of Animal Science curriculum on life skills, students collectively responded more frequently with “a lot” or “a great deal” to increases in skills learned from being in the Animal Science degree (Table 4). Students reported that they increased their ability to recognize a sound argument and appropriate use of evidence. One key outcome targeted by the Animal Science major is to improve critical thinking of undergraduate students. Critical thinking is important to personal and professional success for students and faculty (Vermeulen and Schmidt, 2008). Teachers that enhance critical thinking allow students to apply their knowledge in future classes or careers (Abou-Zaid, 2014). A students’ understanding does not simply stop at that class; but should broaden skills in school and life experiences (Abou-Zaid, 2014). Of employers surveyed, 93% stated “a demonstrated capacity to think critically, communicate clearly, and solve complex problems is more important than [a candidate’s] undergraduate major”. Furthermore, employers also wish to have a greater emphasis on critical thinking because, while 87% of students believe college experiences prepare them to think critically, only 6% of graduates demonstrate significant abilities in critical thinking (Facione, 2010). Furthermore, studies have shown a direct correlation between the ability

to think critically and academic success (Groschner et al., 2010) due to the ability to postulate answers and not only learn facts.

Of the five specific skills related to communication and critical thinking, the ability to write documents in discipline-appropriate style and format had the least amount of responses for “a great deal” and “a lot” noting that the program did not do an effective job in teaching students this skill (Table 4). Additionally, students ranked their attitude of preparing and giving an oral presentation lower. Students that are able to effectively communicate their scientific ideas are more desirable graduate and workforce candidates. Animal Science can further work to improve written and oral communication by first familiarizing students with scientific literacy and writing styles. Giving direct instruction on how to read and analyze articles has been shown to significantly increase scientific literacy (Krontiris-Litowitz, 2013). It may be beneficial to emphasize to students the importance of communication in their future career.

At the completion of their degree program, students were enthusiastic about Animal Science and confident in their future success in an Animal Science career (n=244; 96%, Table 5).

A majority of students (n=191) noted improved ability to apply principles of Animal Science to new problems by “a lot” or “a great deal (Table 6). They also noted improved ability to use a systematic reasoning to approach problems (n=189). Another learning outcome targeted by the Animal Science program is to improve student’s ability to use a systems-based approach to problem-solving. In respect to enhanced problem-solving skills, students are able to handle more stress and unknowns in a classroom atmosphere because they are able to pull previous knowledge and use various ways to

come to a solution (Prevost and Lemons, 2016). Overall, there has been a positive effect of curriculum among semesters analyzed showing professors have provided students with the necessary attributes to be successful past their time as an undergraduate student in Animal Science.

Post-test Curriculum Questions:

Students also completed a post-test on concepts which instructors of core courses felt were important for students to understand at graduation (Table 7). Students' scores were significantly lower in some discipline areas compared to others ($P < 0.05$). These differences may be due to several factors. Students should take a basic physiology course as well as general animal industry and biology course early in their undergraduate career. The next course the majority of students take in the sequence is a basic animal nutrition course. Students generally take an advanced animal breeding and genetics and animal reproductive physiology course during their third or fourth year. Students were not asked which courses they had completed at the time of the survey. Therefore, some students may have been currently enrolled or not yet enrolled in the advanced courses at the time of taking the survey. Students scored similarly ($P = 0.688$) in their percent correct in the areas of meat science and nutrition. Concepts learned in these courses are applied in advanced courses which may result in students being better able to retain material. The number of correct student responses was lower ($P < 0.05$) percent correct in the areas of animal genetics and physiology. These concepts require students to have a deeper understanding of basic sciences. As noted earlier, students found it difficult to understand the need to learn basic science concepts from biology and chemistry. Having a limited foundation in these may decrease students' ability to be successful in some

courses. In addition, due to course sequencing students may not have completed these courses. Due to the fact that questions were submitted by individual instructors, there may be variation in difficulty of questions submitted. While the student's scores varied among subject, semester had no effect (P = 0.10).

The significant differences of genetics and physiology compared to other subjects may be due to course concepts being more difficult. Furthermore, the variation in the difficulty of questions was not analyzed among subject. However, results may indicate the need to review genetics and physiology concepts more with students in order to improve overall results.

According to Araz and Sungar (2007) there needs to problem-based learning in genetics courses. Problem-based learning is an approach in science education that focuses on helping students to develop self-directed learning skills (Barrows and Tamblyn, 1980). While many students will excel in the area of their interest, this does not always hold true for genetics courses. Through a hands-on project assigned by Araz and Sungar (2007) students in a genetics course had the opportunity to meet with farmers and apply their knowledge. The performance of students who completed the hands-on project verses those who did not, showed there were strengths to students learning in a classroom setting as well as students learning out on the farm. However, further research is needed to perfect the practice of integrating both methods and which to students each method would apply best (Araz and Sungur, 2007).

Problem-based learning may be used less in science classes due to the amount of curriculum the instructor needs to cover. Factual learning has a distinct right and wrong answer and learning may not allow students to think critically, but instead encourages

memorization. The school of physics has shown that problem-based learning can be successfully integrated and can improve not only science skills but also, group work, personal learning, and communication (Facione, 2010). Problem-based learning whether it be through group projects, field trips, discussions all have positive correlations with students learning and can help them to apply what they learn in other aspects of their life (i.e classes and careers). The Animal Science department may be able to use field trips to various farms and research sites in order to help students apply what they are learning in the classroom. By having pretests and posttests, instructors can address how their students are learning and where there are gaps in knowledge.

Summary

Having results of students across several semesters increased the number of students assessed and helped eliminate any outliers. Because there was no significant difference based on semester, an overall evaluation of the major's learning outcomes could be addressed.

Addressing the strengths of the department will help students in understanding the benefits of the program as they progress through their degree. Current instructors will need to evaluate courses to improve learning outcomes based on results of the post-test. While students are confident in how the Animal Science department has prepared them in certain aspects, a focus on bringing other science backgrounds into use during Animal Science class is not as strong. It has been shown that through hands-on experience students are able to connect knowledge from other subjects such as nutrition, genetics, and physiology (Waddell, 2018). Furthermore, introducing students early on to scientific writing will help with their scientific literacy and competency. It would be recommended

that professors allow hands on work in order to help students better grasp concepts while also giving direct instruction until the topic is adequately understood. Critical thinking can be difficult if there are too many gaps in one's knowledge of a subject, thus thorough teaching through various teaching practices (i.e. group work, lab work, scientific reading) is recommended. In conclusion, students' thoughts and ability to recall what was previously taught has stayed steady through the years and there needs to be a greater focus on the topics of physiology and genetics.

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Table 1. Number of students who completed the Animal Science senior assessment survey and breakdown of major based on responses to Animal Science senior assessment survey

Year	Assessment Completed	Total in Class	Percent Completed	Animal Science Major	Non-Animal Science Major
Fall 2015	46	54	85.2%	54 (100%)	0 (0%)
Spring 2015	46	51	90.2%	46 (90.2%)	5 (9.8%)
Fall 2016	47	49	95.9%	47 (95.9%)	2 (3.1%)
Spring 2016	32	40	80.0%	33 (82.5%)	7 (7.5%)
Fall 2017	39	43	90.7%	35 (81.4%)	8 (8.6%)
Spring 2017	43	46	93.5%	44 (95.7%)	2 (4.3%)
Total	253	283	89.4%	259 (91.5%)	24 (8.5%)

Table 2. Breakdown of GPA based on responses to Animal Science senior assessment survey[1]

YEAR	Below 2.0	2.0-2.50	2.51-3.0	3.01-3.59	3.60-4.0+
Fall 2015	0 (0%)	3 (6.5%)	11 (23.9%)	20 (43.5%)	12 (26.1%)
Spring 2015	1 (2.2%)	4 (6.9%)	14 (30.4%)	16 (34.8%)	11 (23.9%)
Fall 2016	0 (0%)	3 (6.4%)	13 (27.7%)	18 (32.3%)	13 (27.7%)
Spring 2016	0 (0%)	4 (12.5%)	10 (32.0%)	10 (32.0%)	7 (21.9%)
Fall 2017	0 (0%)	5 (13.2%)	14 (36.8%)	13 (34.2%)	6 (15.8%)
Spring 2017	0 (0%)	3 (7.0%)	10 (43%)	15 (34.9%)	15 (34.9%)
TOTAL[2]	1 (0.4%)	21 (8.3%)	72 (28.6%)	92 (36.5%)	64 (25.8%)

[1]No statistical differences in GPA of students across semesters.

[2]N = 252 students

Table 3. Number of students who responded that completion of an Animal Science major improved their understanding based on a five-point Likert scale combined across all 6 semesters[1]

Item	Not at All	Just a Little	Somewhat	A Lot	A Great Deal
Biology and chemistry of the life sciences and application of the principles to animal nutrition, growth, reproduction, genetics and management of animals and their products	3	9	66	136	42
How to develop animal nutrition, growth, reproduction, genetics and management recommendations related to the specific animal or animal product in the career paths related to my selected option	2	6	61	133	54
The terms, facts and concepts of Animal Science	3	0	17	117	119
How ideas we explore in Animal Science classes relate to ideas I have encountered in other classes.	2	9	45	125	75
How ideas we explore in my biology and chemistry classes relate to my Animal Science classes	6	36	77	95	42
How studying Animal Science helps people address real-world issues	3	6	28	119	100

[1]No statistical differences between semesters. Data from all semesters was combined.

Table 4: Number of students who responded that completion of an Animal Science major improved specific skills based on a five-point Likert scale combined across all 6 semesters

Item	Not at All	Just a Little	Somewhat	A Lot	A Great Deal
Critically read articles about issues raised in Animal Science classes.	2	7	43	105	99
Recognize a sound argument and appropriate use of evidence	3	2	41	126	84
Develop a logical argument	3	4	45	108	96
Write documents in discipline-appropriate style and format	5	2	59	100	90
Work effectively with others	2	1	13	94	146
Prepare and give oral presentations	3	4	37	119	93

[1]No statistical differences between semesters. Data from all semesters was combined.

Table 5. Number of students who responded that completion of an Animal Science major improved their attitude based on a five-point Likert scale combined across all 6 semesters

Item	Not at All	Just a Little	Somewhat	A Lot	A Great Deal
Enthusiastic about Animal Science	2	3	7	51	193
Confident that I can be successful in an Animal Science career	4	1	24	75	152
Comfortable working with complex ideas	2	6	35	142	71
Confident in my ability to understand societal and ethical issues related to animals	3	0	27	122	104
Willing to seek help from others (teacher, peers, TA) when working on an academic problem	6	4	41	101	104
Prepare and give oral presentations	3	7	45	115	86

[1]No statistical differences between semesters. Data from all semesters was combined.

Table 6: Number of students who responded that completion of an Animal Science major improved their integration of learning based on a five-point Likert scale combined across all 6 semesters

Item	Not at All	Just a Little	Somewhat	A Lot	A Great Deal
Applying principles of Animal Science to new problems and situations	2	3	60	124	67
Using systematic reasoning in my approach to problems	3	3	61	124	65

[1]No statistical differences between semesters. Data from all semesters was combined.

Table 7. Average Percent Correct on Comprehensive Senior Exit Exam by subject

Subject	Average
Genetics	28.65[a]
Nutrition	71.82[b]
Meat Science	75.15[b]
Physiology	53.38[c]

[1]No statistical differences between semesters. Data from all semesters was combined.

[2]Those not connected by like subscripts were significantly different with a p value < 0.05

Chapter 4. Veterinary Student Case Study Project Leads to Development of Professional Skills

Abstract: At the University of Nebraska-Lincoln, veterinary students enrolled in a nutritional biochemistry course designed their own case studies in groups of 4-5 people. Upon completion of the project, students completed an exit survey ranking items on a 1 (strongly disagree) to 5 (strongly agree) Likert-type scale to analyze the effectiveness of using case studies. A total of 41 students completed the survey. Students indicated that they had a better appreciation for nutrition research after they completed the project (mean = 3.54, SD = 1.21). Students expressed that the nutrition assignment allowed them to apply what they had learned in previous classes to the case study they were presenting (mean = 3.78, SD = 0.91), as well as, allowed them to apply what they were taught in this class to their case study (mean = 4.09, SD = 0.92) . Individuals indicated that the completion of the project did not improve their communication skills (mean = 2.63, SD = 1.01), but did slightly improve their critical thinking skills (mean = 3.29, SD = 0.98). The project objective was to encourage students to connect previous knowledge to new concepts, but the group-work/case study likely had other benefits beyond this one project.

Key words: Case Study, Nutrition, Veterinary student,

Introduction

Veterinarians require a unique combination of medical knowledge and nontechnical skills including empathy, communication skills, and management skills in order to be successful (Lane and Bogue, 2010). Well-structured group projects are known to enhance intellectual and social skills that help prepare students for work outside the classroom. Working as a group post-graduation is very typical in the veterinary industry,

thus practicing these skills in the classroom can help a student's success in their career. According to the National Survey of Student Engagement (2006), positive group experiences can contribute to the student's learning as well as their ability to retain information better and overall classroom success. Group projects have a benefit of allowing professors to assign projects that encompass a majority of the learning objectives of the course. This not only allows students to apply what they have learned throughout the semester and review material but can also serve as an indicator of overall student learning and understanding.

Working in a group not only enhances group-work skills, but also individual skills. When analyzing veterinary student's thoughts on an individual basis vs a group basis on the business side of veterinary medicine, there were a greater number of concepts brought to light by students when they worked in a group vs working alone (Chan and Jackson, 2018). When working in a group, individuals can achieve a more complex way of thinking to identify and understand different concepts that are not apparent when working alone (Chan and Jackson, 2018). Chan and Jackson (2008) found that group work puts an emphasis on learning complex issues due to students to being able to discuss concepts resulting in moving from basic to more complex thinking. In group projects, more complex and challenging projects can be assigned than if the project was going to be completed by an individual (Carnegie, 2014). Students must interact and use other students within their group as a resource to complete the project.

Using case studies to teach promotes critical thinking through active learning (Popil, 2011). Critical thinking is especially important to veterinarians as it allows one to analyze and evaluate a situation before coming to a solution. Through critical thinking, a

veterinarian is able t appropriately assess and diagnosis each patient on an individual basis (Fajt et al., 2009). Case studies offer students a time to use problem solving skills and promote decision making in a "real client" type setting enabling even greater preparation for their career. Using a case study teaching method is an effective tool for active learning that provides students with a variety of important skills in problem solving, critical-reasoning, and analytical skills, which in return, enhances student decision-making, resulting in them becoming better students and veterinarians (Kunselman and Johnson, 2004).

The objective of this project was to evaluate student perceptions of the impacts of completing the case studies on their understanding of course concepts and its impact on skill development.

Materials and Methods:

Course set up and enrollment

Nutritional Biochemistry (VMED 550) is a core class in the curriculum for students in the Professional Program in Veterinary Medicine at the University of Nebraska-Lincoln. The course is offered in an on-campus, traditional lecture format. Course enrollment was 41 students over the two semesters data was collected. Students enrolled in the course were first year veterinary students.

Case Study

Students were required to participate in a group project to design a case study over a topic related to a metabolic disorder or a nutritional deficiency/toxicity. The objectives of this group project were for the groups to demonstrate their understanding of nutrient metabolism as it applies to a specific metabolic disorder by 1) developing a

problem (case study) to be delivered to their peers within the class that stimulates interest in the topic; 2) delivering the case study to their peers in such a way that requires the audience to make decisions; 3) connecting previous course knowledge to new concepts; and 4) challenging their peers to practice higher order problem solving skills. Students allocated to groups of four to five students based upon species of interest. Specifically, at the beginning of the semester students responded to a survey that asked to rank their interest with respect to small (companion) animals, large animals, exotic animals, or mixed species interest. The results of this survey were used to allocate students to their respective groups. The first task for each group was to identify three potential topics related to a metabolic disorder or a nutritional deficiency/toxicity and to submit the topics to the instructor for approval and feedback before moving forward with the project. After identifying their topic of interest and consulting with the professor (by scheduling a face to face meeting), each group was required to develop their case study by preparing power point presentation (minimum 15 minutes containing at least six slides) according to the following guidelines delivered to the students at the introduction of the project: Step 1) Develop the and state the central theme/idea pertaining to the selected problem (disorder); 2) Develop the case study 'story-line' including patient history, signs/symptoms, results of physical examination, diagnosis, treatment plan, and background information related to nutrient metabolism; 3) Develop at least six questions based upon knowledge, comprehension, application, analysis, synthesis, and evaluation; 4) Develop (separately from the presentation) a detailed answer guide to the questions; and 5) Deliver the presentation to their peers in a manner that stimulates discussion.

Students were required to develop and support their chosen topic area with a minimum of three peer-reviewed articles.

The presentation guidelines were designed so that when the presentation (case study) was delivered to their peers in class it would require their peers to make decisions while connecting previous knowledge to new concepts. Furthermore, students were challenged to develop questions that would require their peers within the audience to practice higher order thinking and problem-solving skills. The case study assignment accounted for 10% (50 out of 545 total points) of the course grade and was evaluated based upon the following rubric: 1) Central idea (10 pts.); 2) Development of the 'story-line' (15 pts.); 3) Development of questions (15 pts.); and 4) Question answer guide (10 pts.). In addition, the questions developed by each group for each of the respective case studies were used as the basis for a portion (48%, 60 out of 125 total points) the final course exam

Case Study Evaluation

An evaluation tool was developed to be completed by first year veterinarian students at the completion of their case study group assignment (Appendix 3). Students were given the survey in class to increase the completion percentage. The survey asked demographic information, including ethnicity and gender. In addition, students were asked to respond based on the five point Likert-type scale (5 = strongly agree, 4 = agree, 3 = neither agree nor disagree, 2 = disagree, 1 = strongly disagree) to a series of questions. Survey questions were designed to obtain feedback from students on how the group project pertained to their future career goals, how each student interacted with other members of the group and instructors to complete the project, and how the project

enhanced new and old skills. The procedures of the survey were reviewed and approved by the University of Nebraska-Lincoln's Institutional Review Board (IRB).

Results and Discussion

There were 42 students enrolled in the course among both semesters and 98% of students completed the survey. Similar to the trend seen in recent years in the veterinary industry, the majority (76%) of students were female and only 24% were male. This gender gap has become the norm, as according to the American Veterinary Medical Association, veterinary colleges are made up of about 80% women since 2010 (Burns, 2010).

Case Study and Professor Impacts

A well-described and planned activity enables students to work towards understanding the learning objectives of that assignment. When evaluating the quality and impacts of the assignment (ranked on a 5-point Likert-scale), students noted they were given adequate instruction for the project (mean = 4.07, SD = 0.79) and understood what was expected of them (mean = 4.10, SD = 0.89). In addition, the professor was easy to reach for questions and further instructions (mean = 4.05, SD = 0.89) and overall students enjoyed working with the professor (mean = 3.61, SD = 1.09). Furthermore, they believed that the instructions to present using a PowerPoint was the best way to show their data (mean = 3.90, SD = 1.03). While the rating was lower, students indicated they enjoyed working on the project overall (mean = 3.32; SD = 1.08).

The benefits of group work on learning can be significant, yet an ill designed project can do more harm than good. Through teaching a specific topic, the teacher develops a deeper understanding of the concepts and a greater understanding (Whitman,

1988). A well-designed assignment with clear instructions and thought out groups are more beneficial to student learning and can aid in diligent teamwork and effective collaboration in the project (Carnegie, 2014). Students learn best when they are challenged but are comfortable in what is expected of them and feel their work will be evaluated fairly (Bain, 2004). Clear learning objectives for the students are an important as part of project (Balzer et al., 2015). Having adequate instruction greatly aids in a student's learning.

The overall evaluation process for this project, including the development of the project in consultation with the instructor, the development of different types of questions based on various orders of Bloom's taxonomy, and the use of student developed questions as the basis for a portion of the final exam created an environment where the students were allowed to practice higher order thinking and problem-solving skills. In addition, by utilizing student designed questions as part of the final examination, students were given ownership in the learning and evaluation process.

According to Bassaw et al. (2003), having assignment objectives clear to the students allows a student to know where to direct their focus. A well-described and planned activity enables the student to determine what a particular activity is supposed to accomplished and will benefit a student's learning (McKimm and Swanwick, 2009).

Impacts of Working in Groups

When students answered questions based on working with their classmates, opinions varied (Table 2). When asked if getting the audience (classmates) involved in their presentation for questions at the end furthered their thinking, students moderately agreed that it was beneficial (mean = 3.24, SD 1.11). Students were in general agreement

that their classmates were willing to participate in asking questions post-presentation (mean = 3.98, SD = 1.10). Furthermore, students agreed that they enjoyed working in a group setting (mean = 3.34, SD = 1.13) and that all group members participated equally (mean = 4.07, SD = 1.31).

According to a study conducted by Bene and Bergus (2014), peer teaching benefits both those who are doing the teaching and the peers they are teaching. In particular, peer teaching is a positive strategy for medical schools to engage students as teachers. Through having students teach their peers they have greater motivation to learn the subject due to having to present their information in a clear concise manner. Peets (2009) found the peer teachers spent nearly three times more? time reviewing content that they were going to teach. Furthermore, peer assisted learning, such as the students presenting their case study, relies on the interactions between students in order to successfully fulfill this teaching method. Due to the similar understanding and knowledge of the subject between a student teacher and student learner, the comprehension of facts and understanding enhances the ability to relay information from students to students. Sometimes, the knowledge gap between professors and students can results in a loss or inability to communicate on the same level (Lockspeiser et al., 2008).

Skills Evaluation

Students were asked to evaluate the specific skills earned from this group project (Table 3). Students indicated that they were able to use previous knowledge from class in order to connect it to new concepts (mean = 4.09, SD = 0.92) and concepts from other classes when working on the project (mean = 3.78, SD = 0.91). One student stated, “I enjoyed applying concepts from class to a real-life clinical setting.” Students also agreed

that through the case study project, their understanding of nutrition and metabolic disorders were enhanced (mean = 2.53, SD = 1.43) and that they now have a better appreciation for the importance of nutrition and animal research (mean = 3.54, SD = 1.21). Students agreed that at the completion of the project they were interested in taking more nutrition classes (mean = 3.56, SD = 1.27).

Felder and Brent (1996) indicated that using cooperative (team-based) learning properly in college settings enhances motivation to learn, retention of knowledge, depth of understanding, and appreciation of the subject being taught. The use of group learning in higher education has increased with the goal for students to connect course content to research practices (4, 8). Students specifically stated in the comments section of the survey that they enjoyed the case study because of the "application to real clinical situations in veterinary medicine" and "it helped to solidify ideas and felt important when applying it to a veterinary scenario". It is important for veterinary students to understand nutrition and have the ability to apply their knowledge in a clinical study because many diseases can be influenced by nutrition (Chandler and Takashima, 2014). Diseases including nutrient-sensitive, diet induced, and feed management problems are major problems that veterinarians are asked to address each day (Chandler and Takashima, 2014). Nutrition knowledge is often used in practice and developing that skill in school holds great importance, but it not often fully incorporated into the curriculum.

Individuals had mixed opinions when asked if this project would be beneficial for their future career (mean = 3.05, SD = 1.12). Students were neutral regarding effects of completion of the project on their communication skills (mean = 2.63, SD = 1.01), but indicated slight improvement in their critical thinking skills (mean = 3.29, SD = 0.98).

Case study presentations and audience involvement can allow students to enrich their own learning and skills due to the process of active learning (Jones, 2014). Properly developed group projects, students can develop professional skills needed in their career, such as decision-making skills, critical thinking, communication, and active learning (Millis, 2014). However, veterinary students are different from other students and have been shown to focus their skills only on academics and depriving other aspects of their life causing a rise in anxiety and depression among the veterinary discipline (Hafen et al., 2013). Traits such as perfection and conscientiousness are high among these students and group work does not always come easy. Due to students feeling competitive with one another and not finding time to work together, this can result in negative effects on group work (Meyer-Parson et al., 2017). However, in a professional career in veterinary medicine the ability to explain concepts and diagnoses to their clientele is important. Group work and presentations may help to develop those skills where veterinary students may be less comfortable.

Lastly, students indicated moderate improvement in their ability to support their ideas with research (mean = 3.76, SD = 1.04) and how to present research topics (mean = 3.02, SD = 1.01) with one student expressing they liked "being able to dig into the research and gain a good understanding of a clinical case and now after completing the project, understanding the condition." Students tend to learn best in a case study scenario due to their drive to solve the problem and have the abilities to do so (Ewell, 1997). Group projects are becoming more popular in veterinary school as the shift towards competent traits, such as communication and teamwork become more of a focus. As this shift continues to occur, collaborative learning is challenging veterinary students to no

longer focus on individualistic skills but to work as a group. Veterinary students may benefit from continuous group work in order to improve professional skills and recognize the benefits of working with others.

Summary

The case study allowed students to apply their knowledge to a clinical based scenario. First-year students in a professional veterinary medicine program lack experience in a clinical setting. This project gave students an opportunity to use theoretical knowledge gained in the course and critical thinking skills to practice aspects of a problem-oriented approach to arrive at a clinical diagnosis and potential nutritional interventions for a hypothetical patient. Students agreed the project related to their future career goals (mean = 3.05, SD = 1.12) in the sense of the project mimicking a clinical setting. Students are able to develop skills that they can apply to the veterinary profession. The need for a deeper understanding of animal nutrition and the ability to apply and explain these concepts will be beneficial when working with future clients.

Overall, students had positive comments about the case study project and its connection to the veterinary industry, while also stating they enjoyed working in groups. This project helped solidify core concepts that were taught in the class and allowed students to apply knowledge from previous classes into their case studies. Veterinary students may be less comfortable with working in a group project setting so providing more opportunities to work in a group on projects with clearly defined expectations and learning outcomes can be beneficial. These skills allow students to become more confident in themselves and in completing complex tasks within a group setting. By

learning how to collaborate with each other, students will be more prepared for their careers.

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Table 1. Student evaluation of the case study design and professor impacts in a group case study while enrolled in veterinary nutritional biochemistry

Item	**Mean**	**SD**
I was given adequate instruction for the project.	4.07	0.79
I understood what was expected out of me for this project.	4.10	0.89
The teacher was easy to reach for questions and further instruction.	4.05	0.89
I feel that PowerPoint was the best way to present my case study.	3.90	1.03
I enjoyed working with your professor.	3.61	1.09
I enjoyed working on this project.	3.32	1.08

Ranked on a scale of 1-5: 1 = strongly disagree, 2 = disagree, 3 = neutral, 4 = agree, 5 = strongly agree

Table 2. Effects of student participation in a group case study while enrolled in veterinary nutritional biochemistry

Item	**Mean**	**SD**
Getting the audience involved, helped further your thinking on your case.	3.24	1.11
My classmates were willing to actively participate in the project during question time.	3.98	1.10
The members in my group equally contributed to the completion of this project.	4.07	1.31
I enjoyed working in a group.	3.34	1.13

Ranked on a scale of 1-5: 1 = strongly disagree, 2 = disagree, 3 = neutral, 4 = agree, 5 = strongly agree

Table 3. Impact of group case study from veterinary nutritional biochemistry on the development of a student's professional skills and future decisions.

Item	**Mean**	**SD**
I had to improve my communication skills to complete this project.	2.63	1.00
Completing the project improved my critical thinking skills.	3.29	0.98
I better understand how to support my ideas with research.	3.76	1.04
I have a better understanding of how to present research.	3.02	1.01
After completing this project, I have a better appreciation for nutrition.	3.54	1.21
I can see how completing this project will help with my future education.	3.40	1.16
Completion of this project will be beneficial to my future career.	3.05	1.12
I would like to take more nutrition classes.	3.56	1.27
This project enhanced my understanding of nutrition and metabolic disorders.	2.53	1.43
I am more interested in a career with research after completing this course.	2.38	1.20
I applied the concepts we learned in class when completing this assignment.	4.09	0.92
I was able to use previous knowledge to connect it to new concepts.	3.78	0.91

Ranked on a scale of 1-5: 1 = strongly disagree, 2 = disagree, 3 = neutral, 4 = agree, 5 = strongly agree

Chapter 5. Effects of Energy Restriction During Gilt Development on Litter Performance, Gut Microbiome and Milk Peptides

Abstract: Gilt longevity has been assessed in an ongoing experiment at the University of Nebraska–Lincoln (including 17 batches of gilts; n = 90 gilts/batch). In the current experiment, this model is used to evaluate sow and progeny growth performance, microbiota of progeny and sow, and milk peptides derived from gilts developed on various dietary treatments. For the analysis described herein, batch 16 gilts were the focal point. During the development period, batch 16 gilts (n = 56, 8 gilts/pen) were fed 3 dietary treatments: 1) Control - formulated to NRC (2012) specifications (CTL); 2) Restricted - 20% energy restriction via addition of 40% soy hulls; (RESTR); and, 3) CTL plus - addition of crystalline amino acids equivalent to the SID Lys:ME of the RESTR diet (CTL+). Following breeding (230 d of age), all gilts were fed standard gestation and lactation diets formulated to meet NRC (2012) specifications. Data on litter (including weaning weight and birth weight) and dam performance (backfat and lactation feed intake) was recorded. No difference was observed on progeny growth performance based on diet ($P > 0.05$). With respect to the dam, pre-backfat ($P = 0.003$), backfat loss ($P = 0.024$), and lactation feed intake ($P = 0.021$) were all affected by dietary treatments. Fecal samples were collected from both piglets (n = 152) and dams (n = 38) on d 0 and d 14 post-farrowing for analysis of microbial population. Microbial population was affected by diet, day, and pig type (piglet v. sow) ($P = 0.016$, $P < 0.001$, $P < 0.001$, respectively). From d 0 to 14 individual piglet microbiome increased in richness (species count) and variation (diversity of species) ($P < 0.001$); however, there was little difference among variation and richness in sow microbiome from d 0 to 14. Furthermore, individual piglet

microbiome had less richness and variation when compared to sow's ($P < 0.001$). Among the dietary treatments, there were 89 differently abundant genre that were represented among 13 different phyla. Milk samples were collected from batch 16 gilts (n = 3/treatment) on d 0 and 14 post-farrowing for peptidomic analysis and showed a clustering effect by day. Gut microbiome, and milk peptidomics could provide valuable insight to the health of offspring and future impact of diet. Overall, developmental diet of the dam may impact progeny microbiome and milk peptide composition.

Key words: Microbiome, Peptidomics, Sow Nutrition

Introduction

Due to previous research done by Miller et al. (2011), where it was observed that feeding a restricted energy diet to gilts during their developmental stages lead to increased longevity, further research was conducted on the progeny of 14 reps of sows being fed similar diets as explained by Miller et al. (2011), to see how diet affected them. Barnett et al. (2017), reported on the progeny effects across 14 reps, finding that not only did a restricted energy diet increase sow longevity, but it may also increase progeny weaning weight. Most studies today solely focus on altering the diet of sows during lactation and gestation and there is little research on the effects of diet prior to gestation on progeny.

Research has shown that perinatal nutrition can greatly affect the physiology of the neonate, specifically on health programming (Jacobi and Odle, 2012). Maternal nutritional state before conception can have major effects on both the growth and the development of the offspring. Furthermore, maternal nutrition prior to conception can

also affect the long- and short-term health of offspring (Adane et al., 2018). Altering the maternal diet will alter the dams gut microbiome and in-turn alter that of the offspring. The development of an organism's microbiota is quite complex and evolves throughout life. The initial population of an offspring's microbiome is developed through their interaction with the dam by their type of delivery and later through breastmilk (Milani et al., 2017). The neonate's gut is then comprised of numerous bacteria that play a key role in metabolism, nutrition, and immunity throughout the rest of its life (Milani et al., 2017).

Through breastfeeding, neonates are able to acquire their needs for both nutritional and immune support. As diet is altered for the dam, milk nutrient composition may also be altered. Maternal milk contains many peptides that regulate the infant's metabolism (Aydin, 2017). Small, medium, and large peptides in breast milk serve a multitude of biological functions. Peptides within breastmilk aid in the development of the small intestine and innate immunity, as well as digestion, growth, and development (Aydin, 2017). Maternal diet has many effects on its offspring whether it be in utero or through lactation. Therefore, our working hypothesis is that alterations in the composition of the diet of the dam during the gilt development phase may contribute to changes in milk peptide composition and progeny gut microbial populations contributing to effects on the growth the health of the piglets. To test our hypothesis, this experiment was designed to evaluate the effects of energy restriction on sow and litter performance, dam and progeny gut microbial populations, and milk peptides.

Materials and Methods

The University of Nebraska, Lincoln Animal Care and Use Committee approved all animal care and handling procedures used in this experiment. The Experiment was carried out at the University of Nebraska Swine Research Center (Mead, NE).

Animals and Experimental Design

Batch 16, parity 1 gilts (n = 63) were randomly allotted to a dietary treatment (3 treatments, 8 gilts/pen) during their developmental period (d 120 to 230 of age). Genetics of gilts used were sires that were Yorkshire, and dams that were ½ DNA Yorkshire, ¼ DNA Landrace, and ¼ WXL line 452 (a combination of genetic lines from a previous selection experiment for increased litter size (Hsu and Johnson, 2014)). Gilts were housed in a temperature-controlled room and were given ad libitum access to water. Gilts were fed in a 3-phase feeding regimen in which phase 1 and 2 were 42 d, and phase 3 was 26 d. At 230 d of age, gilts were bred through artificial insemination and moved to individual gestation crates where they were all fed a common diet to meet the requirements of a gestating sow (NRC, 2012). At d 109 of gestation the sows were moved to farrowing crates and fed a common diet to meet the requirements of a lactating sow (NRC, 2012).

Dietary Treatments

Diet ingredients and nutrient composition are presented in Table 1 for the experimental diets (fed from age 120 d to 230 d) and Table 2 for the common gestation (fed from 230 d of age to d 109 of gestation) and lactation (fed from d 109 of gestation until piglets are weaned) diet. Experimental diets were given ad libitum and varied based on energy content. Dietary treatments included the following: 1) Control (CTL; formulated to 2012 NRC requirements) 2) restricted (RESTR; containing 40% soy hulls

and 20% energy restricted) and 3) Control Plus (CTL+; containing an addition of crystalline amino acids equivalent to the SID Lys:ME of the RESTR diet).

Data and Sample Collection

During lactation sows were given ad libitum and feed disappearance to obtain average daily feed intake (ADFI) was statistically analyzed based on individual sow. When the gilts were moved to farrowing crates (d 109 of gestation), pre-backfat (pre-BF) was measured using Aloka 500V real-time ultrasound instrument equipped with a 3.5-MHz, 17-cm linear transducer (Corometrics Medical System, Inc., Wallingford, CT) and pre-body weight (pre-BW) weight was recorded. After farrowing, at the time progeny were weaned (d 21 post-farrowing), gilt backfat loss and post-BW were observed and recorded as described previously. Piglets were weaned at d 21 post-farrowing. Sow litter performance was recorded for total number born (TNB) and number weaned (NW). Piglets that were cross fostered were moved to a farrowing crate with a gilt on the same treatment as that from which it was derived. All piglets' birth weight (BiW) and weaning weight (WW) were collected to measure progeny performance based on developmental diet. Milk samples (n = 18) and fecal samples (n= 38) of the sows were collected on d 0 and 14 post-farrowing from. Oxytocin (1 to 2 mL) was administered in the vulva to facilitate milk letdown. Piglets (n = 152; 4/litter) from the gilts were randomly selected and fecal samples were collected on d 1 and 14 post-farrowing. Fecal and milk samples were frozen at -20 ºC for subsequent analyses.

Gut Microbiome Analysis

Frozen fecal swabs were removed from the -20°C freezer and DNA extraction was performed on each individual fecal sample (190 pigs x 2 time points). utilizing the

Mag Bind Soil DNA Isolation Kit (Omega Bio-tek, Norcross, GA) according to the manufacture's purification protocol with several modifications; the fecal swabs were dipped in sterile 2.0 mL Safe-Lock tube (Eppendorf, North America, Inc., USA) containing 0.5 g silica beads (Scientific Asset Management, Basking Ridge, NJ) and 300 µL of SLX-Mlus Buffer to help wash the swab; bead-beating was completed in a Tissue Lyser (QIAGEN Inc., Valencia, CA) at a speed of 20 beats/s for 10 min. Centrifugation at (5000 × G) was performed on the samples and the supernatant was removed and placed into sterilized 1.5 mL tubes (Fisherbrand, Fischer Scientific, USA). The remaining protocol was performed according to the manufacturer's instructions. DNA quality was then determined using gel electrophoresis and the remaining DNA was stored at -20°C and later used for further analysis.

The elution plate and bacterial primer plate developed by Samohda Fernando Lab were thawed on ice and a PCR plate to be tested was made which included, Master mix : Terra Buffer (12.5ul) + polymerase (0.5ul) + H20 (9.0ul) mixed and added to Primer (1 uL) and DNA (2ul). After adding reagents to the plate seal with a plate cover**,** Veriti 96-well thermocycler (Life Technologies™, Carlsbad, CA) was used to perform the amplifications. The PCR conditions for the reaction were 3 min at 98°C for 1 cycle. followed by 25 cycles of 30 s at 98°C, 30 s at 55°C, and 45 s at 68°C, with a final cycle of 4 min at 68°C. After the amplification, the PCR products were resolved in a 2% agarose gel. The samples were run through gel electrophoresis at 120 V for 60 min to verify size and that amplification had occurred.

Samples were later normalized using the NGS normalization 96-well kit (Norgen Biotek corp. ON, Canada) following the protocol that came with the kit. Plates were

individually “pooled” and then purified. To purify the samples the NucleoSpin Gel and PCR CleanUp kit (Takara Bio, Mountain View, CA) was used according to “PCR clean-up” manufacturer instructions. Once purified dna was quantified with a Denovix kit and reader (DeNovix, Wilmington, DE) and steps were followed according to manufacturer protocol. Libraries were shown to be eligible for a 2 nM sequencing run using a V3 kit with an MiSeq Illumina Sequencing platform using a 500 cycle V2 kit (Illumina, Inc., USA) according to the manufacture’s protocol.

Milk sample preparation and LC-MS/MS analysis

The samples were prepared according to Dingess et al. (2017). Briefly cOmplete EDTA-free protease inhibitor cocktail (Millipore Sigma) was added to an aliquot of 500 µL of sample. The samples were centrifuged at 1,500 × g for 10 min to remove the fat droplets. The liquid phase was pipetted into a new tube and proteins precipitated by adding TCA to 10%. The protein pellet was removed by centrifugation at 16,000 × g for 10 min. The supernatant was transferred to a new tube and cleaned up using SPE cartridges (Sep-Pak C18 1 cc Vac Cartridge, 50 mg, Waters). The eluted peptides were dried down and analyzed by LC-MS/MS using a RSLCnano system (ThermoFisher Scientific) coupled to a Q-Exactive HF mass spectrometer (ThermoFisher Scientific). The samples were first injected onto a trap column (Acclaim PepMap™ 100, 75 µm × 2 cm, ThermoFisher Scientific) for 2.8 min at a flow rate of 5 µL/min, 1.5% acetonitrile, 0.2% formic acid before switching in-line with the main column. Separation was performed on a C18 nano column (Acquity UPLC® M-class, Peptide CSH™ 130A, 1.7 µm 75 µm x 250 mm, Waters Corp) at 260 nL/min with a linear gradient from 5-32% over 35 min. The LC aqueous mobile phase contained 0.1% (v/v) formic acid in water

and the organic mobile phase contained 0.1% (v/v) formic acid in 80% (v/v) acetonitrile. Mass spectra for the eluted peptides were acquired on a Q Exactive HF mass spectrometer in data-dependent mode using a mass range of m/z 375–1500, resolution 120,000, AGC target 3×10^6, maximum injection time 60 ms for the MS1 peptide measurements. Data-dependent MS2 spectra were acquired by HCD as a Top12 experiment with a normalized collision energy (NCE) set at 28%, AGC target set to 1×10^5, 15,000 resolution, intensity threshold 1×10^5 and a maximum injection time of 250 ms. Dynamic exclusion was set at 20 sec and the isolation window set to 1.6 m/z.

Statistical analysis

Data was analyzed in JMP 12 (Cary, NC) and used LSMEANS Differences with Tukey-HSD Adjustment was used for all growth analysis. $P < 0.05$ was considered significant, non-significant factors were dropped and the model was rerun. When analyzing treatment effect, backfat loss (BF), weight loss, lactation feed intake, and WW were included in the model as response variables and treatment as the fixed effect and total number weaned as a covariate. In analyzing WW, Lactation feed intake, NW, and BF loss were fixed effects and WW was the response variable. Birth weight was then analyzed with TNB as a covariate and treatment as a response variable and birthweight being the fixed effect. Lastly, Pre-BF was analyzed with birth weight, number born alive and TNB as response variables and Pre-BF and the fixed variable.

Data for microbiome analysis was analyzed through R package "stats" (version 2.15.3). Chimeras of the DNA were removed to prevent skewed results. An ASV table was generated through R, using Dada2 and a phylogenic tree in Motur in which the two were merged and a mapping profile self-created through excel for variables of interest

were included. To analyze differential abundance of bacteria between sow v piglet and diet DeSeq was used to calculate a negative binomial.

Milk peptides were analyzed with Mascot v 2.6.1 (Matrix Science, UK) which was searched using the common contaminants database cRAP (123 entries, www.theGPM.org) and the Uniprot reference proteome database for *Sus scrofa* (retrieved on 20191122, 40,702 entries) with a fragment ion mass tolerance of 0.060 Da and a parent ion tolerance of 10.0 PPM, assuming no enzyme. Methionine oxidation and deamidation (Asn, Gln) were set as variable modifications. A false discovery rate of 1% was used for confident peptide identification. Progenesis QI-P (Waters, version 4.1) was used to quantify the peak area from the peptide ions identified using the database search.

Results

Sow and Progeny Performance

Growth performance data are presented in Table 3a. Treatment had an effect on the parity 1 sows lactation intake ($P = 0.0291$) in which sows that were on the CTL+ diet consumed the least amount of feed (2.09 kg/d), while the RESTR sows consumed the most (2.4 kg/d). Furthermore, treatment also had an effect on BF loss ($P = 0.0240$) in which the CTL+ sows lost more (-0.476mm) backfat compared to sows on the RESTR diet (-0.162mm). Interestingly, treatment did not have a direct effect on WW ($P = 0$ .9820) or NW ($P = 0.9267$), but WW was effected by BF loss ($P = 0.0160$), lactation feed intake ($P = 0.0002$), and NW ($P < 0.0001$) which diet did have an effect.

Treatment did not have an effect on birth weight ($P = 0.3712$) or TNB ($P=$ 0.8279), but did effect Pre-BF ($P = 0.0003$) in which CTRL had the greatest (2.06mm)

and RESTR had the lest (1.64mm). Pre-BF, however, did have an effect on birth weight ($P = 0.0374$), number born alive ($P = 0.0134$), and TNB ($P = 0.027$).

Gut Microbiome

Fecal samples of 4 piglets per sow (n = 252) and all sows (n = 63) were analyzed through 16s DNA gene sequencing. Microbial population was affected by diet, day, and pig type (piglet v. sow) ($P = 0.016$, $P < 0.001$, $P < 0.001$, respectively). Among the three diets of both sow and piget, there were 89 differently abundant genre that were represented among 13 different phyla. Overall individual piglet microbiome had less richness and variation when compared to sow's ($P < 0.001$).

Phylum level

As seen in Figure 1, the phylum diversity among diets is very similar in samples obtained from both sows and piglets. Figure 1 shows, Firmicutes were most abundant among all three diets, followed by proteobacteria and Bacteroidetes. Furthermore, RESTR and CTRL+ followed the same pattern over time with Firmicutes increasing, and Bacteroidetes decreasing. Little change was observed at the phylum level over time in the CTRL diet. From d 0 to 14 individual piglet microbiome increased in richness (species count) and variation (diversity of species) ($P < 0.001$); however, there was little difference in variation and richness in sow microbiome from d 0 to 14. At the phylum level, piglet fecal samples, proteobacteria was 4fold greater in the piglet and tenericutes was 3.3-fold greater in the sow (Table 4). With respect to the CTL diet, phylum tenericutes and Firmicutes had a greater abundance when compared to the RESTR diet.

Genus Level

As seen in Figure 2, the Simpson index, which has a greater focus on the dominant bacteria, indicates the diversity present within a species. With greater species richness and evenness, diversity increases. A similar trend was observed with the Shannon index where abundance of the bacteria is not weighted. According to diet effect, CTL was significantly more diverse than both the RESTR and CTL+. Interestingly, fecal samples from progeny s on the CTL+ diet contained no bacteria that was not present in both the CTL and RESTR diet. However, fecal samples from progeny and sows the CTL diet had 14 bacteria present at the genus level that were not present in the RESTR diet including prevotella; whereas, clostridium and lactobacillus were observed to be present in fecal samples from the RESTR diet and not in the CTL diet. CTL and RESTR had distinct genus that were greater ($P < 0.05$) in the gut when compared to CTL+ (Table 5a and 5b). When comparing the sow and piglet on the genus level the sow had many more types of bacteria upregulated than the piglet. Some bacteria that were upregulated in the sow compared to the piglet were prevotella, lachnospirae, and Bifidobacterium, whereas the piglet had an upregulation of streptococcus, enterococcus, and Bacteroides. Among all the bacteria found Tyzzerella, Bifidobacterium, and Erysipelotrichaceae were the bacteria that were the greatest indicators of which diet was being consumed.

Peptidomics

A total 885 peptides were identified using database search of the Uniprot Sus scrofa. These peptides were derived from a total of 68 proteins (Table 6). As seen in Figure 3, the PCA plot shows how D1 and D14 samples cluster separately based on their similarity of protein abundance regardless of diet. Because samples are not true

replicates, the fold change was calculated from the sum of the peak area from samples D1 and samples D14.

Discussion

The analysis of the data obtained when these developmental diets were employed was to evaluate whether altering energy intake during growth would have an effect on sow longevity (Miller et al., 2011). Data obtained from 14 batches of sows lead to the conclusion that energy restricted gilts have greater longevity (Miller et al., 2011). Because of these previous observations, further analysis was conducted to see if developmental diet affected sow progeny. Among those 14 batches observed, it was also noted that gilts on an energy-restricted diet may result in parity 1 offspring with a greater weaning weight (Barnett et al., 2017). The idea of restricting energy during gilt development is based on the premise that restricting metabolizable energy intake should result in decreased fat deposition, but not affect muscle accumulation (Miller et al., 2011). With developmental diet possibly affecting offspring performance as seen in previous data analysis by Barnett et al. (2017), evaluating microbial populations of offspring and milk peptides could help us understand what may be causing the differences in litter and progeny performance among sows developed on different dietary treatments.

Growth performance

While weaning weight was not affected by treatment in rep 16 this may be due to the number of pigs analyzed in a single rep. However, although treatment had no direct effect on weaning weight, it did affect lactation feed intake, backfat loss, total number of piglets born, and pre-backfat which are all variables that have been correlated to birth

weight and weaning weight (Amdi et al., 2013). During fetal development, there is adaptation by the fetus to the nutritional status of the mother through fetal-placental physiology, hormones, and metabolic modifications (Fleming et al., 2018). Sows that are over- or under-fed reduce fetal-blood flow and stunt their growth (Wu et al., 2004). Furthermore, Andreas et al. (2014), reported that the body mass index has an impact on milk composition, yet altering diet to modulate breastmilk composition is still an understudied topic. While there is little research on altering milk composition due to body mass index, data from this study supports that altering a sows feed intake and nutrient composition of the feed will affect sow body condition, milk composition (in regards to milk peptides), and progeny microbial DNA, thus affecting progeny growth.

In the current experiment, it was observed that sows on the RESTR diet had greater lactation feed intake and less backfat loss. When sows are pregnant, they are limit-fed to prevent obesity, which can lead to other negative consequences such as farrowing problems and poor lactation intake (Ramonet et al., 2000). However, similar to the current study, several other studies have concluded that diets with higher fiber vs energy will improve sow and litter performance during lactation (Reese, 1997; Veum et al., 2009). During lactation many sows become catabolic in order to me the demands of their litter (Strathe et al., 2017a); however, with increased feed intake during lactation sows are better able to meet the energy and nutrient requirements through their milk output resulting in healthier, heavier pigs (Strathe et al., 2017b). Feeding sows a diet that allows them to meet their energy demands while lactating has direct effects on piglet performance.

Gut Microbiome

Breastmilk contains a complex variety of bioactive compounds, including proteins, peptides, lipids, micronutrients, nucleotides, hormones, growth factors, immunomodulatory agents, human milk oligosaccharides (HMO), and microbes (Le Doare et al., 2018). In studies conducted on humans, it has been shown that the gut microbiome alters during pregnancy based on body mass index and weight gain (Collado et al., 2008). Lower quantities of Bifidobacterium species and overall microbial diversity have been reported in obese, pregnant women compared to those that are at the appropriate weight (Santacruz et al., 2010). Similar reports have been found on that of breastmilk of obese women. Cabrera-Rubio et al. (2010), reported that the greater gestational weight gain the lower the milk microbial diversity. These studies align with the current study in which sows and their piglets from the CTL+ group had the least amount of diversity in their microbiome.

The relationship between diet, microbiome, and health have been long studied and prenatal nutrition affects all aspects of the neonate, not just growth and development. Maternal nutrition, even before the conception of the fetus, can have profound long- and short-term effects on the fetus (Adane et al., 2018). Bacteria that originates in the intestines of the sow reach the offspring in-utero through the placenta and then through the maternal milk when the infant is nursing (Macpherson et al., 2017). Continuing, vaginally delivered infants come in contact with the mothers vaginal and fecal microbiota as opposed to those delivered through caesarean-section; therefore, vaginally delivered neonates have a gut that right away is inhabited by vagina-associated microbes, including *Lactobacillus* and *Prevotella* (Milani et al., 2017).

Among all three diets, Firmicutes and Bacteroidetes were of the most abundant phyla which is consistent with other studies focusing on piglet microbial population (Wen et al., 2018). Studies have shown that a 1:1 ratio of Firmicutes and Bacteroidetes are related to a healthy body mass index, whereas a low presence of Bacteroidetes is often seen in obese subjects (Hildebrandt et al., 2009). As seen in the current study, sows fed the CTL+ diet had a greater body fat and both the sows and piglets had less Bacteroidetes. Data suggests that you can have a high-fat diet without being obese and still obtain a healthy Bacteroidetes:Firmicute ratio (Hildebrandt et al., 2009).

The third most prevalent phyla in each diet groups microbiome was Proteobacteria. *Proteobacteria* is known to be the most diverse bacterial phylum and are obligate anaerobic bacteria. Proteobacteria can be associated with opportunistic pathogens, such as *Escherichia coli, Salmonella,* and *Campylobacter*. High abundances of *Proteobacteria* have been associated with an unbalance in the gut commonly associated with metabolic or inflammatory disorders (Moon et al., 2018). Bradley and Pollard (2017), stated that although Firmicutes and Bacteroidetes dominated the gut microbiome, proteobacteria contribute to the functional variation of the host.

Milk Peptides

Milk has many bioactive components that affect all aspects of the body. When milk is ingested proteins are broken down and bioactive peptides are released (Park and Nam, 2015). These bioactive peptides from milk can have beneficial effects such as antimicrobial, antioxidative, and immunomodulatory activities (Park and Nam, 2015). While milk is the primary nutrient source of neonates, the degradation of milk proteins release peptides that have different affects from those of the parent protein (Nielsen et al.,

2017). The majority of these functional peptides are derived from casein and whey proteins (Nielsen et al., 2017). In this study, it was observed that milk peptides clustered based on similarity of protein abundance by day regardless of diet. Peptides that are found within breastmilk are released from native proteases during fermentation and digestion (Dallas et al., 2015). Among bioactive peptides present in milk there are several groups that target different aspects of the body. Opioid peptides bind to opioid gut receptors where they alter gastrointestinal motility, antimicrobial peptides can inhibit pathogen growth, and angiotensin-converting enzyme peptides are absorbed into the blood and can lower blood pressure (Nielsen et al., 2017, Chabance et al., 1998). Furthermore, whey protein derived peptides are absorbed more quickly than those derived from Casein while also having different metabolic functions (Boirie et al.,1997). Milk proteins also alter based on lactation stage to fit the needs of the neonate for development and growth (Tari et al., 2019). Once the milk is digested many bioactive peptides are released within the gut and bloodstream (Barbe et al., 2014). This is similar to what was seen in the peptides observed in this study where there was a difference based on time. Time most likely was the only effect because sows were not on different diets during gestation or lactation to cause a diet effect. According to a study by Tari et al. (2019), where piglets were fed milk formula with altered levels of casein and whey, the piglet was affected in areas of metabolic and physiological responses. Interestingly, results showed that regardless of the level of casein in the formula fed to piglets, there was significantly higher average daily gain, average feed intake, and feed efficiency when compared to piglets fed a formula only containing whey. These findings provide a better understanding of milk peptides and how day or diet will affect the composition.

Hopefully through these findings, more research can be conducted on sow milk to better understand how to alter milk to best fit the needs of a growing piglet. Further research is required to conclude how sow diet affects milk peptides.

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Table 1. Ingredient composition and nutrient analysis of diets (as-fed basis) fed to developing gilts d 123 to 230. Diets consisted of a control (CTL), restricted (RESTR), or control plus (CTL+). Phase 1 and 2 were each 6 wk in duration and phase 3 was 4 wk in duration.

	Phase 1			Phase 2			Phase 3		
Item	**CTL[1]**	**RESTR[2]**	**CTL+[3]**	**CTL**	**RESTR**	**CTL+**	**CTL**	**RESTR**	**CTL+**
Ingredient, %									
Corn	72.52	39.59	70.38	76.32	43.17	74.66	80.13	47.16	78.60
Soybean Meal	21.53	17.79	23.35	17.66	14.13	19.00	13.79	10.05	15.00
Soybean Hulls	-	40.00	-		40.00	-		40.00	-
Beef Tallow	3.00	-	3.00	3.00	-	3.00	3.00	-	3.00
Dicalcium phosphate	1.37	1.72	1.37	1.46	1.80	1.46	1.54	1.89	1.54
Limestone	0.68		0.68	0.66		0.66	0.64		0.64
Sodium Chloride	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin Premix[4, 5]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mineral Premix[6]	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
L-Lysine	-	-	0.15	-	-	0.15	-	-	0.15
Methionine	-	-	0.05	-	-	0.05	-	-	0.05
Threonine	-	-	0.09	-	-	0.09	-	-	0.09
Tryptophan	-	-	0.03	-	-	0.03	-	-	0.03
Calculated composition:									
ME, kcal/kg	3406	2705	3408	3408	2706	3410	3410	2707	3412
Lys, g/kg	0.7	0.7	0.86	0.61	0.61	0.76	0.51	0.51	0.66
CP, %	13.72	12.68	14.34	12.36	14.41	12.81	11.01	12.79	11.41
P, %	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60
Ca, %	0.67	0.71	0.68	0.67	0.72	0.68	0.68	0.73	0.68
Lys/ME (g/Mcal)	2.06	2.57	2.53	1.78	2.24	2.22	1.50	1.87	1.93

[1] Control diet (CTL) was formulated to meet 2012 NRC requirements for developing gilts.

[2]Energy restricted diet (RESTR) was 20% restricted in energy with increased fiber.
[3]Control Plus (CTL+) contained an addition of crystalline amino acids equivalent to the SID Lys:ME of the RESTR diet.
[4] Provided per kilogram of diet for phase 1 and 2: 5,500 IU of Vitamin A, 550 IU of Vitamin D_3, 30 IU of Vitamin E, 4.40 IU of Vitamin K, 33.00 mg of Niacin, 22.05 mg of Pantothenic Acid, 11.00 mg of Riboflavin, and 33.00 g of Vitamin B_{12}
[5]Provided per kilogram of diet for phase 3: 6,600 IU of Vitamin A, 600 IU of Vitamin D_3, 66 IU of Vitamin E, 4.40 IU of Vitamin K, 33.00 mg of Niacin, 22.05 mg of Pantothenic Acid, 11.00 mg of Riboflavin, and 22.05 g of Vitamin B_{12}, 550 mg of Choline Chloride, 1.65 mg of Folic Acid, 0.22 mg of Biotin
[6] Provided per kilogram of diet: 10.50 mg of Copper Sulfate Pentahydrate, 0.26 mg of Calcium Iodate, 127.50 mg of Ferrous Sulfate, 30.00 mg of Manganese Oxide, 0.30 mg of Sodium Selenite, 127.50 mg of Zinc Sulfate, 226.03 mg of Calcium Carbonate.

Table 2. Ingredient composition and nutrient analysis of diets (as-fed basis) fed during gestation and lactation.

Item:	Gestation[1]	Lactation[2]
Ingredient, %		
Corn	77.25	65.68
Soybean Meal, 47.5 % CP	16.00	27.50
Tallow	3.00	3.00
Dicalcium Phosphate	1.90	2.33
Limestone	0.93	0.60
Salt	0.50	0.50
Vitamin Premix[3]	0.25	0.25
Trace Mineral	0.15	0.15
Phytase	0.02	-
Calculated composition		
ME (kcal/kg)	2605	2536
CP, %	11.74	15.75
Lys ana	0.56	0.85
Total P, %	0.67	0.80
Ca, %	0.87	0.90

[1]Gestation diet was fed from the day of breeding until farrowing.
[2]Lactation diet was fed beginning at farrowing through d 21 post-farrowing, sows were put immediately back on gestation diet at d 21 post-farrowing.
[3]Provided per kilogram of diet for phase 3: 6,600 IU of Vitamin A, 600 IU of Vitamin D_3, 66 IU of Vitamin E, 4.40 IU of Vitamin K, 33.00 mg of Niacin, 22.05 mg of Pantothenic Acid, 11.00 mg of Riboflavin, and 22.05mg of Vitamin B_{12}, 550 mg of Choline Chloride, 1.65 mg of Folic Acid, 0.22 mg of Biotin

Table 3. Effects of feeding gilts control (CTL; n = 19), restricted (RESTR; n = 25), or control plus (CTL+; n = 18) diets on gilt and litter performance. Means in same row not connected by the same letter differ ($P < 0.05$).

	CTL	RESTR	CTL+	*SEM*	*P*-Value
Pre-BF of gilts, mm[1]	2.06[a]	1.64 [b]	2.02 [a]	0.107	0.0003
BF loss of gilts, mm[2]	0.30[ab]	0.16[a]	0.47[b]	0.351	0.024
Progeny BiW, kg[3]	18.11[a]	18.02 [a]	19.08 [a]	0.577	0.371
Litter WW, kg[4]	70.65 [a]	71.35 [a]	71.15 [a]	1.720	0.918
Lactation Feed Intake, kg	5.00[ab]	5.30[a]	4.60[b]	0.106	0.021
Number Weaned	11.63 [a]	11.72 [a]	11.72 [a]	0.312	0.982

[1]Gilt backfat at d 109 of gestation (pre-BF)
[2]Gilt backfat at weaning (post-BF; 21 d post-farrowing)
[3] average total litter birth weight (BiW)
[4] average adjusted weaning weight (WW) of litter

Table 4. Phylum that are significantly more prevalent in the piglets based on diet

Bacteria	baseMean	log2FoldChange	*P*-Value	Upregulated
Tenericutes	4.609	1.054	0.0002	RESTR
Spirochaetes	8.185	1.434	< 0.001	CTL
Firmicutes	4044.9	0.955	< 0.001	RESTR

Table 5a. Top 10 Genus that are significantly more prevalent in the piglets from a sow that were on the CTRL diet vs the other experimental diets

Bacteria	baseMean	log2FoldChange	*P*-Value
Pyramidobacter	2.438	1.953	< 0.001
Acidaminococcus	3.580	1.799	< 0.001
Treponema.2	8.402	1.742	< 0.001
Mitsuokella	2.934	1.221	< 0.001
Dialister	4.392	1.144	< 0.001
Prevotella.9	3.791	1.064	< 0.001
Syntrophococcus	1.838	0.957	< 0.001
Proteus	6.498	0.869	0.006
Eggerthella	2.175	0.779	0.001
Slackia	1.311	0.777	< 0.001

Table 5b. Top 10 Genus that are significantly more prevalent in the piglets from a sow that were on the RESTR diet vs the other experimental diets

Bacteria	baseMean	log2FoldChange	*P*-Value
Faecalibacterium	1.194	0.438	0.020
Ruminiclostridium	1.201	0.472	0.015
Veillonellaceae.UCG.001	1.234	0.472	0.021
Bacillus	1.205	0.482	0.012
Lachnospiraceae.FCS020.group	1.495	0.493	0.014
Prevotella.7	1.688	0.595	0.007
Ruminiclostridium.9	4.713	0.644	0.016
Peptoniphilus	4.257	0.665	0.014
Aerococcus	1.537	0.677	<0.001
Arcanobacterium	2.303	0.711	0.004

Figure 1. Phylum diversity of bacteria present in sows and piglets based on diet A (CTL) B (RESTR) and C (CTL+)

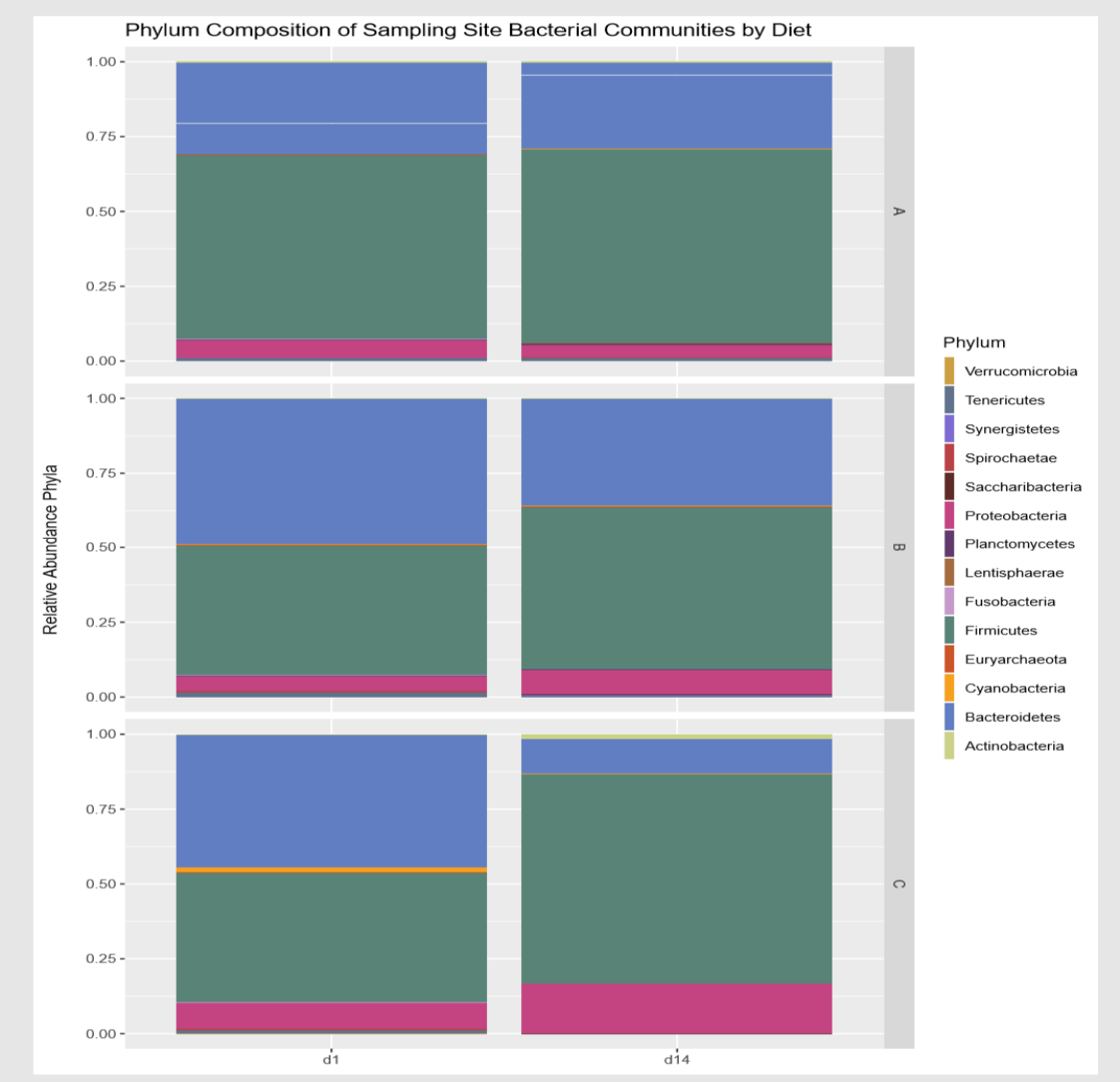


Figure 2. The community diversity of bacteria present in the pig based on diet A (CTL), B (RESTR), and C (CTL+).

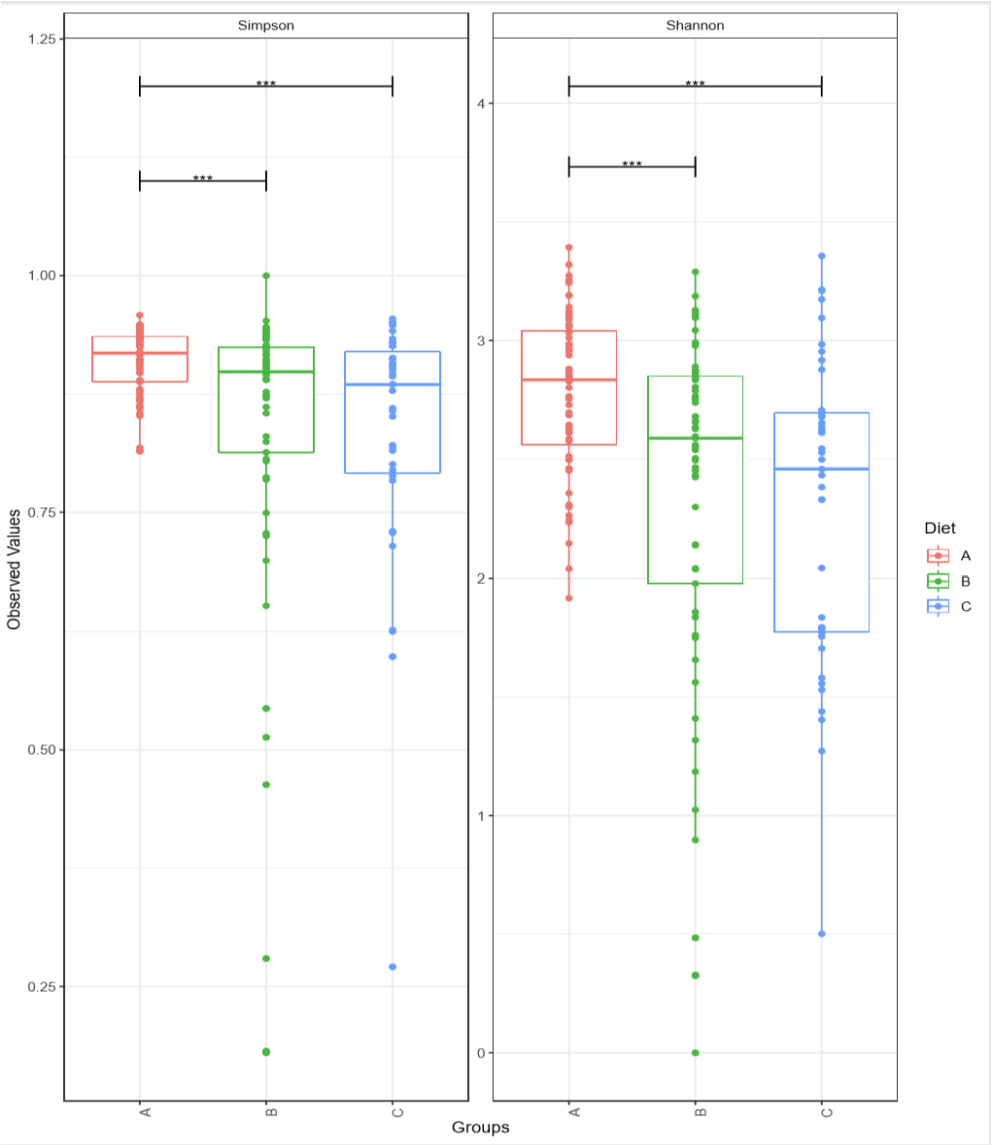


Table 6. list of all 68 proteins found within the sows milk among all dietary treatments and days.

Accession[1]	Peptide count	Confidence score	Mass	Description[2]
sp|P04119|LACB_PIG	49	3056.3	19721	sp|P04119|LACB_PIG Beta-lactoglobulin-1A/1C OS=Sus scrofa OX=9823 PE=1 SV=4
sp|P27917|APOC3_PIG	7	308.08	10697	sp|P27917|APOC3_PIG Apolipoprotein C-III OS=Sus scrofa OX=9823 GN=APOC3 PE=1 SV=2
sp|P39035|CASA1_PIG	51	2228.38	24133	sp|P39035|CASA1_PIG Alpha-S1-casein OS=Sus scrofa OX=9823 GN=CSN1S1 PE=2 SV=1
sp|P39036|CASA2_PIG	13	690.73	27553	sp|P39036|CASA2_PIG Alpha-S2-casein OS=Sus scrofa OX=9823 GN=CSN1S2 PE=2 SV=1
sp|P39037|CASB_PIG	117	4758.1	25933	sp|P39037|CASB_PIG Beta-casein OS=Sus scrofa OX=9823 GN=CSN2 PE=1 SV=1
sp|Q4PLW0|PLIN2_PIG	26	1245.79	50164	sp|Q4PLW0|PLIN2_PIG Perilipin-2 OS=Sus scrofa OX=9823 GN=PLIN2 PE=2 SV=1
tr|A0A0B4J2J8|A0A0B4J2J8_PIG	1	52.7	30696	tr|A0A0B4J2J8|A0A0B4J2J8_PIG Uncharacterized protein OS=Sus scrofa OX=9823 GN=FCGR2B PE=4 SV=1
tr|A0A2C9F376|A0A2C9F376_PIG	114	4664.89	25762	tr|A0A2C9F376|A0A2C9F376_PIG Beta-casein OS=Sus scrofa OX=9823 GN=CSN2 PE=1 SV=1
tr|A0A286ZHY0|A0A286ZHY0_PIG	1	53.44	162132	tr|A0A286ZHY0|A0A286ZHY0_PIG LAM_G_DOMAIN domain-containing protein OS=Sus scrofa OX=9823 GN=COL16A1 PE=4 SV=1
tr|A0A286ZIL9|A0A286ZIL9_PIG	1	55.44	129802	tr|A0A286ZIL9|A0A286ZIL9_PIG LAM_G_DOMAIN domain-containing protein OS=Sus scrofa OX=9823 GN=COL18A1 PE=1 SV=1

Accession[1]	Peptide count	Confidence score	Mass	Description[2]
tr|A0A286ZJT3|A0A286ZJT3_PIG	1	34.32	85799	tr|A0A286ZJT3|A0A286ZJT3_PIG Uncharacterized protein OS=Sus scrofa OX=9823 GN=SUPT20H PE=4 SV=1
tr|A0A286ZK97|A0A286ZK97_PIG	2	97.13	13492	tr|A0A286ZK97|A0A286ZK97_PIG Ig-like domain-containing protein OS=Sus scrofa OX=9823 PE=4 SV=1
tr|A0A286ZMZ2|A0A286ZMZ2_PIG	5	212.55	57691	tr|A0A286ZMZ2|A0A286ZMZ2_PIG RRM domain-containing protein OS=Sus scrofa OX=9823 GN=CPSF6 PE=1 SV=1
tr|A0A286ZR49|A0A286ZR49_PIG	4	144.7	17489	tr|A0A286ZR49|A0A286ZR49_PIG Uncharacterized protein OS=Sus scrofa OX=9823 GN=PMAP-23 PE=3 SV=1
tr|A0A286ZV96|A0A286ZV96_PIG	1	34.74	17003	tr|A0A286ZV96|A0A286ZV96_PIG Uncharacterized protein OS=Sus scrofa OX=9823 GN=C2orf40 PE=4 SV=1
tr|A0A286ZYT5|A0A286ZYT5_PIG	4	178.84	48447	tr|A0A286ZYT5|A0A286ZYT5_PIG Uncharacterized protein OS=Sus scrofa OX=9823 GN=CEACAM1 PE=1 SV=1
tr|A0A286ZZL9|A0A286ZZL9_PIG	1	38.95	48776	tr|A0A286ZZL9|A0A286ZZL9_PIG Uncharacterized protein OS=Sus scrofa OX=9823 GN=ABCB6 PE=4 SV=1
tr|A0A287A5B4|A0A287A5B4_PIG	1	44.19	69264	tr|A0A287A5B4|A0A287A5B4_PIG BRO1 domain-containing protein OS=Sus scrofa OX=9823 GN=PDCD6IP PE=1 SV=1
tr|A0A287A142|A0A287A142_PIG	2	127.55	8579	tr|A0A287A142|A0A287A142_PIG Apelin OS=Sus scrofa OX=9823 GN=APLN PE=4 SV=1
tr|A0A287AA85|A0A287AA85_PIG	1	44.01	41916	tr|A0A287AA85|A0A287AA85_PIG Hydroxycarboxylic acid receptor 2 OS=Sus scrofa OX=9823 GN=HCAR2 PE=3 SV=1

Accession[1]	Peptide count	Confidence score	Mass	Description[2]
tr|A0A287AAI3|A0A287AAI3_PIG	2	90.72	9450	tr|A0A287AAI3|A0A287AAI3_PIG Non-histone chromosomal protein HMG-17 OS=Sus scrofa OX=9823 GN=HMGN2 PE=1 SV=1
tr|A0A287AIG3|A0A287AIG3_PIG	8	491.59	40269	tr|A0A287AIG3|A0A287AIG3_PIG Uncharacterized protein OS=Sus scrofa OX=9823 GN=B4GALT1 PE=1 SV=1
tr|A0A287AKL0|A0A287AKL0_PIG	75	4490.01	13956	tr|A0A287AKL0|A0A287AKL0_PIG Serum amyloid A protein OS=Sus scrofa OX=9823 PE=3 SV=1
tr|A0A287ALQ2|A0A287ALQ2_PIG	1	31.06	77715	tr|A0A287ALQ2|A0A287ALQ2_PIG AMP-binding domain-containing protein OS=Sus scrofa OX=9823 GN=ACSL6 PE=1 SV=1
tr|A0A287AME1|A0A287AME1_PIG	1	111.08	59030	tr|A0A287AME1|A0A287AME1_PIG Uncharacterized protein OS=Sus scrofa OX=9823 GN=CANX PE=1 SV=1
tr|A0A287APD5|A0A287APD5_PIG	1	41	80224	tr|A0A287APD5|A0A287APD5_PIG Long-chain-fatty-acid--CoA ligase 3 OS=Sus scrofa OX=9823 GN=ACSL3 PE=1 SV=1
tr|A0A287AZG3|A0A287AZG3_PIG	1	37.5	124295	tr|A0A287AZG3|A0A287AZG3_PIG Alpha-mann_mid domain-containing protein OS=Sus scrofa OX=9823 GN=MAN2A1 PE=1 SV=1
tr|A0A287B0N8|A0A287B0N8_PIG	1	25.12	88572	tr|A0A287B0N8|A0A287B0N8_PIG Uncharacterized protein OS=Sus scrofa OX=9823 GN=UNC5D PE=4 SV=1
tr|A0A287B5M2|A0A287B5M2_PIG	1	94.66	131943	tr|A0A287B5M2|A0A287B5M2_PIG Uncharacterized protein OS=Sus scrofa OX=9823 GN=MUC4 PE=4 SV=1
tr|A0A287B8B0|A0A287B8B0_PIG	1	38.85	42941	tr|A0A287B8B0|A0A287B8B0_PIG Perilipin OS=Sus scrofa OX=9823 GN=PLIN3 PE=1 SV=1

Accession[1]	Peptide count	Confidence score	Mass	Description[2]
tr|A0A287BC37|A0A287BC37_PIG	1	70.15	110218	tr|A0A287BC37|A0A287BC37_PIG Histone-lysine N-methyltransferase OS=Sus scrofa OX=9823 GN=SETD1A PE=4 SV=1
tr|A0A287BEK5|A0A287BEK5_PIG	7	447.19	50801	tr|A0A287BEK5|A0A287BEK5_PIG Uncharacterized protein OS=Sus scrofa OX=9823 GN=SL44-1 PE=4 SV=1
tr|A0A287BET3|A0A287BET3_PIG	1	51.3	54939	tr|A0A287BET3|A0A287BET3_PIG Uncharacterized protein OS=Sus scrofa OX=9823 GN=ATP5F1B PE=1 SV=1
tr|A0A287BIE8|A0A287BIE8_PIG	4	222.87	43490	tr|A0A287BIE8|A0A287BIE8_PIG Uncharacterized protein OS=Sus scrofa OX=9823 GN=IST1 PE=1 SV=1
tr|A0A287BJ88|A0A287BJ88_PIG	1	71.45	17688	tr|A0A287BJ88|A0A287BJ88_PIG Transcription factor BTF3 OS=Sus scrofa OX=9823 GN=BTF3 PE=1 SV=1
tr|A0A287BPB0|A0A287BPB0_PIG	1	40.85	192844	tr|A0A287BPB0|A0A287BPB0_PIG Protein kinase domain-containing protein OS=Sus scrofa OX=9823 GN=WNK2 PE=4 SV=1
tr|C3VVV8|C3VVV8_PIG	1	25.63	12065	tr|C3VVV8|C3VVV8_PIG Prothymosin alpha OS=Sus scrofa OX=9823 GN=PTMA PE=1 SV=1
tr|F1RFI1|F1RFI1_PIG	1	47.14	49420	tr|F1RFI1|F1RFI1_PIG Elongation factor Tu OS=Sus scrofa OX=9823 GN=TUFM PE=1 SV=1
tr|F1RGR9|F1RGR9_PIG	5	237.59	63260	tr|F1RGR9|F1RGR9_PIG SEA domain-containing protein OS=Sus scrofa OX=9823 GN=MUC1 PE=1 SV=2
tr|F1RIG4|F1RIG4_PIG	2	96.26	102490	tr|F1RIG4|F1RIG4_PIG Uncharacterized protein OS=Sus scrofa OX=9823 GN=CLSTN1 PE=1 SV=3

Accession[1]	Peptide count	Confidence score	Mass	Description[2]
tr|F1RL72|F1RL72_PIG	1	44.12	26514	tr|F1RL72|F1RL72_PIG Homeobox domain-containing protein OS=Sus scrofa OX=9823 GN=CDX1 PE=4 SV=3
tr|F1RMJ1|F1RMJ1_PIG	1	40.58	87190	tr|F1RMJ1|F1RMJ1_PIG P/Homo B domain-containing protein OS=Sus scrofa OX=9823 GN=FURIN PE=3 SV=1
tr|F1RPA3|F1RPA3_PIG	2	79.22	43051	tr|F1RPA3|F1RPA3_PIG G-protein coupled receptor family C group 5 member B OS=Sus scrofa OX=9823 GN=GPRC5B PE=4 SV=3
tr|F1RQB0|F1RQB0_PIG	30	2254.19	59100	tr|F1RQB0|F1RQB0_PIG Uncharacterized protein OS=Sus scrofa OX=9823 GN=BTN1A1 PE=1 SV=3
tr|F1RQW2|F1RQW2_PIG	1	38.32	192315	tr|F1RQW2|F1RQW2_PIG Complement C4-A isoform 1 preproprotein OS=Sus scrofa OX=9823 GN=C4A PE=1 SV=2
tr|F1RRY2|F1RRY2_PIG	1	65.23	35017	tr|F1RRY2|F1RRY2_PIG Nucleophosmin isoform 3 OS=Sus scrofa OX=9823 GN=NPM1 PE=1 SV=3
tr|F1RT83|F1RT83_PIG	4	182	32409	tr|F1RT83|F1RT83_PIG Uncharacterized protein OS=Sus scrofa OX=9823 GN=SDCBP PE=1 SV=3
tr|F1RTH4|F1RTH4_PIG	1	42.14	70696	tr|F1RTH4|F1RTH4_PIG E74-like factor 4 (Ets domain transcription factor) OS=Sus scrofa OX=9823 GN=ELF4 PE=2 SV=3
tr|F1RVB2|F1RVB2_PIG	11	522.79	20953	tr|F1RVB2|F1RVB2_PIG Kappa-casein OS=Sus scrofa OX=9823 GN=CSN3 PE=3 SV=3
tr|F1RX36|F1RX36_PIG	3	136.74	72568	tr|F1RX36|F1RX36_PIG Fibrinogen alpha chain OS=Sus scrofa OX=9823 GN=FGA PE=1 SV=3

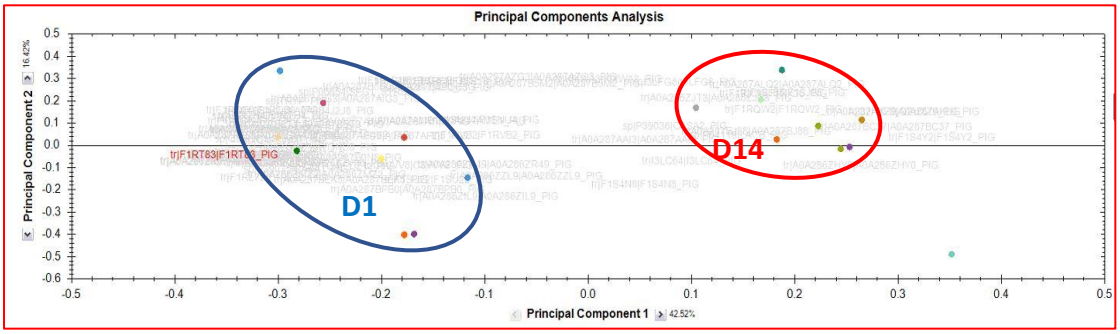
Accession[1]	Peptide count	Confidence score	Mass	Description[2]
tr|F1S4N8|F1S4N8_PIG	1	33.22	71142	tr|F1S4N8|F1S4N8_PIG Vitrin isoform 6 OS=Sus scrofa OX=9823 GN=VIT PE=4 SV=3
tr|F1S4V2|F1S4V2_PIG	1	31.42	45597	tr|F1S4V2|F1S4V2_PIG Uncharacterized protein OS=Sus scrofa OX=9823 GN=MTA3 PE=4 SV=3
tr|F1S4Y2|F1S4Y2_PIG	1	30	217503	tr|F1S4Y2|F1S4Y2_PIG Nucleic_acid_bd domain-containing protein OS=Sus scrofa OX=9823 GN=NCOA6 PE=4 SV=3
tr|F1S5A6|F1S5A6_PIG	6	428.65	73597	tr|F1S5A6|F1S5A6_PIG Sodium-dependent phosphate transport protein 2B isoform X1 OS=Sus scrofa OX=9823 GN=SLC34A2 PE=1 SV=3
tr|F1S029|F1S029_PIG	1	48.59	59286	tr|F1S029|F1S029_PIG Lysophosphatidylcholine acyltransferase 1 OS=Sus scrofa OX=9823 GN=LPCAT1 PE=1 SV=1
tr|F1SB42|F1SB42_PIG	1	46.01	69328	tr|F1SB42|F1SB42_PIG FERM domain-containing protein OS=Sus scrofa OX=9823 GN=EZR PE=1 SV=2
tr|F1SEY8|F1SEY8_PIG	102	6714.42	81587	tr|F1SEY8|F1SEY8_PIG Polymeric immunoglobulin receptor OS=Sus scrofa OX=9823 GN=PIGR PE=1 SV=2
tr|F1SFZ5|F1SFZ5_PIG	9	453.14	36084	tr|F1SFZ5|F1SFZ5_PIG Mucin-15 isoform a OS=Sus scrofa OX=9823 GN=MUC15 PE=1 SV=3
tr|F1SJB5|F1SJB5_PIG	1	47.69	38720	tr|F1SJB5|F1SJB5_PIG Annexin OS=Sus scrofa OX=9823 GN=ANXA1 PE=1 SV=3
tr|F1SMX1|F1SMX1_PIG	1	40.57	29601	tr|F1SMX1|F1SMX1_PIG Uncharacterized protein OS=Sus scrofa OX=9823 GN=C2orf72 PE=1 SV=3

Accession[1]	Peptide count	Confidence score	Mass	Description[2]
tr|F1SNU4|F1SNU4_PIG	1	50.28	20409	tr|F1SNU4|F1SNU4_PIG Uncharacterized protein OS=Sus scrofa OX=9823 GN=LOC100736951 PE=1 SV=1
tr|F1SU22|F1SU22_PIG	1	30.15	51630	tr|F1SU22|F1SU22_PIG Uncharacterized protein OS=Sus scrofa OX=9823 GN=UXS1 PE=4 SV=3
tr|I3LC64|I3LC64_PIG	1	38.06	62656	tr|I3LC64|I3LC64_PIG Uncharacterized protein OS=Sus scrofa OX=9823 GN=ECM1 PE=1 SV=2
tr|I3LFG8|I3LFG8_PIG	1	35.53	101197	tr|I3LFG8|I3LFG8_PIG E3 ubiquitin-protein ligase RNF216 isoform X1 OS=Sus scrofa OX=9823 GN=RNF216 PE=4 SV=2
tr|K7GND8|K7GND8_PIG	1	32.35	51870	tr|K7GND8|K7GND8_PIG Clusterin OS=Sus scrofa OX=9823 GN=CLU PE=1 SV=2
tr|K7GNX7|K7GNX7_PIG	1	52.64	72090	tr|K7GNX7|K7GNX7_PIG Transporter OS=Sus scrofa OX=9823 GN=SLC6A14 PE=3 SV=1
tr|K9IWA3|K9IWA3_PIG	1	54.97	51474	tr|K9IWA3|K9IWA3_PIG V-type proton ATPase subunit S1 OS=Sus scrofa OX=9823 GN=ATP6AP1 PE=2 SV=1
tr|Q2HXZ9|Q2HXZ9_PIG	137	7998.35	14502	tr|Q2HXZ9|Q2HXZ9_PIG Serum amyloid A protein OS=Sus scrofa OX=9823 GN=SAA3 PE=1 SV=1

[1] unique identifier assigned to the protein by FASTA database

[2]Name of Protein exclusive of the assigned accession

Figure 3. Principle component analysis of D 1 and D 14 milk peptides.



Chapter 6. Effects of feeding a Lactobacillus fermentation product during Late Gestation on Litter Performance, Milk Analysis, and Gut Microbiome[1]

Abstract: With growing litters and new veterinary feed directives, pork producers are searching for ways to maintain profitability and increased pig performance. In the current experiment a Lactobacillus fermentation product supplement was top dressed to a gestating sow's feed to evaluate its effect on sow and progeny performance, milk nutrients, and gut microbiome. During the late gestation (d 81 of gestation) parity 4 sows (n = 30; sires that were ¾ Yorkshire, 1/8 Landrace, and 1/8 WXL line 452 (a combination of genetic lines from a previous selection experiment for increased litter size (Hsu and Johnson, 2014)).) were allotted to 3 dietary treatments including a lactobacillus fermented product and continued on the diet until farrowing. Dietary treatments included the following: 1) Control – Formulated to NRC (2012) specifications (CTL; n = 10); 2) Recommended – 6 g of the lactobacillus product/sow/d (REC; n = 10); and 3) Excess – 1.5 times the recommended value, 9 g of lactobacillus product/sow/d (EXC; n = 10). Data on litter (weaning weight, birth weight, number born alive, and stillborn) and dam performance (lactation feed intake) was recorded. No dietary treatment effects were observed on progeny weaning weight ($P = 0.190$), birth weight ($P = 0.483$), or number of stillborn ($P = 0.342$). Interestingly, diet did have an effect on preweaning mortality in which piglets from sows on the REC diet had a higher rate than those on the EXC diet ($P = 0.037$). Milk samples were collected from sows (n =10/treatment) on d 0 and 14 post-farrowing for micronutrient analysis. Day had an effect on many more micronutrients analyzed when compared to diet effect ($P < 0.05$). The only nutrient effected by diet was

milk urea nitrogen in which it was highest in EXC (P = 0.011). Furthermore, Fecal samples were collected from piglets (4 piglets/sow, n = 120) d 0 and d 14 post-farrowing and on sows d 80 of gestation and d 1 and d 14 post-farrowing for analysis of microbial population. Sows displayed a more even distribution among its taxonomy, as well as a greater number of species when compared to the piglet. From d 0 to 14 individual piglet microbiome began to cluster more towards the sow's microbiome, whereas, there was little difference among variation and richness in sow microbiome from d 80 of gestating to d 0 and 14 post-farrowing. Furthermore, there were no different genus or upregulated genus in sows on d 80 before starting treatment, compared to the sows d 1 and d 14 post farrowing. In conclusion, supplementation of a Lactobacillus fermentation product may affect sow and litter performance, milk nutrients, and gut microbiome, but further research needs to be conducted.

Key words: Microbiome, Milk Analysis, Sow Nutrition

Introduction

As pork producers continue to select for sows with larger litter numbers, there needs to be a focus on sow nutrition in order for sows to wean healthier, larger piglets (Hsu and Johnson, 2014). With there being more veterinary feed directives in place, producers are turning towards supplements to maintain the health and growth efficiency of their herd (Lamoreaux, 2019). Supplementing an animal with a product that can boost their immune system while maintaining or enhancing growth is ideal.

A key role in piglet health is the nutrition of its sow and how that effects milk output and gut microbiome. In the current study, sows were fed a commercially available Lactobacillus fermentation product that is said to provide a nutritive source made with

organic compounds that benefit the growth of beneficial digestive bacteria in order to help restore and maintain healthy intestinal microflora of the digestive tract. The supplement fed in this study is similar to that of a prebiotic due to it enhancing the growth of beneficial bacteria, but also that of a probiotic due to it being made from a type of bacteria (Liao and Nyachoti, 2017; Gibson and Roberfroid, 1995). However, the Lactobacillus fermentation product used in this study is neither classified as a probiotic or prebiotic. This product is non-viable, and due to extensive processing to stabilize the strain it allows exposure of the cell wall to the digestive bacteria.

While there is no up to date research on this supplement, pre- and probiotics have been shown to help balance the microbiome of pigs when used as a feed additive at correct levels (Liao and Nyachoti, 2017). Also, because the highest cost within a swine production is feed costs summing up to 2/3 of production, including feed additives to increase feed efficiency greatly attracts pork producers (Patience, 2012). Current research has shown that lactobacillus is the main genera in both the proximal and distal region of the gastrointestinal tract in pigs (Veizaj-Delia et al., 2012) Lactobacilli has been shown to have benefits in newborn piglets and sows, in which sows had increased production performance and piglets had increased average daily gain and reduced population of Clostridium sp. (Liu et al., 2014; Wang et al., 2014).

Therefore, it was hypothesized that sows fed a Lactobacillus fermentation product would have a more balanced gut microbiome which may result in beneficial effects for progeny health and growth.

Materials and Methods

The University of Nebraska, Lincoln Animal Care and Use Committee approved all animal care and handling procedures used in this experiment. The Experiment was carried out at the University of Nebraska Swine Research Center (Mead, NE).

Animals and Experimental Design

Batch 17, parity 4 sows (n = 30) were randomly allotted to a dietary treatment (3 treatments, 10 sows per treatment) during late gestation (d 80 of gestion to farrowing). Genetics of sows used were sires that were Yorkshire, and dams that were ½ DNA Yorkshire, ¼ DNA Landrace, and ¼ WXL line 452 (a combination of genetic lines from a previous selection experiment for increased litter size (Hsu and Johnson, 2014)). Sows were all housed in separate gestion and fed a common diet up to day 80 of gestation to meet the requirements of a gestating sow (NRC, 2012). On day 81 of gestation sows were allotted and started on 1 of the 3 diets for the trial to begin. At d 109 of gestation the sows were moved to farrowing crates and once farrowed they were fed a common diet to meet the requirements of a lactating sow (NRC, 2012).

Dietary Treatments

Diet ingredients and nutrient composition are presented in Table 1 for the experimental diets (fed from d 81 of gestation to farrowing) and for the common gestation (fed from d 1 of gestation to d 80) and lactation (fed from d 1 of farrowing until piglets are weaned) diet. Experimental diets were fed at a level of 1.8 kg a day until day 90 of gestation, in which they then went up to 2.7 kg a day and varied based on the amount of Lactobacillus fermentation product (Culbac-dry; TransAgra International, Inc., Storm Lake, Iowa). Dietary treatments included the following: 1) Control (CTL; formulated to 2012 NRC requirements) 2) Recommended (REC; containing

recommended value of 6 g/feeding) and 3) Excess (EXC; containing 1.5 more than recommended value at 9 g/feeding).

Data and Sample Collection

During lactation sows were given ad libitum and feed disappearance to obtain average daily feed intake (ADFI) was statistically analyzed based on individual sow. When the sows were moved to farrowing crates (d 109 of gestation), pre-backfat (pre-BF) was measured using Aloka 500V real-time ultrasound instrument equipped with a 3.5-MHz, 17-cm linear transducer (Corometrics Medical System, Inc., Wallingford, CT) and pre-body weight (pre-BW) weight was recorded. After farrowing, at the time progeny were weaned (d 21 post-farrowing), gilt backfat loss and post-BW were observed and recorded as described previously. Piglets were weaned at d 21 post-farrowing. Piglets were not cross-fostered. All piglets' birth weight (BiW) and weaning weight (WW) were recorded as measures of progeny performance based on experimental diet. Milk samples (n = 30) of the sows were collected on d 0 and 14 post-farrowing and fecal samples (n = 30) were collected from sows on d 80 of gestation and d 1 and d 14 post-farrowing. Oxytocin (1 to 2 mL) was administered in the vulva to facilitate milk letdown. Piglets (n = 120; 4/litter, 2 male/ 2 female) from the sows were randomly selected and fecal samples were collected on d 1 and 14 post-farrowing. Fecal and milk samples were frozen at -20 ºC for subsequent analyses.

Milk analysis

Frozen milk vials containing approximately 30ml of sample were overnighted to Eastern Laboratory Services (Medina, OH) for micronutrient analysis and free fatty acid analysis.

Gut Microbiome Analysis

Frozen fecal swabs were removed from the -20°C freezer and DNA extraction was performed on each individual fecal sample (190 pigs x 2 time points). utilizing the Mag Bind Soil DNA Isolation Kit (Omega Bio-tek, Norcross, GA) according to the manufacture's purification protocol with several modifications; the fecal swabs were dipped in sterile 2.0 mL Safe-Lock tube (Eppendorf, North America, Inc., USA) containing 0.5 g silica beads (Scientific Asset Management, Basking Ridge, NJ) and 300 µL of SLX-Mlus Buffer to help wash the swab; bead-beating was completed in a Tissue Lyser (QIAGEN Inc., Valencia, CA) at a speed of 20 beats/s for 10 min. Centrifugation at (5000 × G) was performed on the samples and the supernatant was removed and placed into sterilized 1.5 mL tubes (Fisherbrand, Fischer Scientific, USA). The remaining protocol was performed according to the manufacturer's instructions. DNA quality was then determined using gel electrophoresis and the remaining DNA was stored at -20°C and later used for further analysis.

The elution plate and bacterial primer plate developed by Samohda Fernando Lab were thawed on ice and a PCR plate to be tested was made which included, Master mix : Terra Buffer (12.5ul) + polymerase (0.5ul) + H20 (9.0ul) mixed and added to Primer (1 uL) and DNA (2ul). After adding reagents to the plate seal with a plate cover**,** Veriti 96-well thermocycler (Life Technologies™, Carlsbad, CA) was used to perform the amplifications. The PCR conditions for the reaction were 3 min at 98°C for 1 cycle. followed by 25 cycles of 30 s at 98°C, 30 s at 55°C, and 45 s at 68°C, with a final cycle of 4 min at 68°C. After the amplification, the PCR products were resolved in a 2%

agarose gel. The samples were run through gel electrophoresis at 120 V for 60 min to verify size and that amplification had occurred.

Samples were later normalized using the NGS normalization 96-well kit (Norgen Biotek corp. ON, Canada) following the protocol that came with the kit. Plates were individually "pooled" and then purified. To purify the samples the NucleoSpin Gel and PCR CleanUp kit (Takara Bio, Mountain View, CA) was used according to "PCR clean-up" manufacturer instructions. Once purified dna was quantified with a Denovix kit and reader (DeNovix, Wilmington, DE) and steps were followed according to manufacturer protocol. Libraries were shown to be eligible for a 2 nM sequencing run using a V3 kit with an MiSeq Illumina Sequencing platform using a 500 cycle V2 kit (Illumina, Inc., USA) according to the manufacture's protocol.

Statistical analysis

Data for sow and litter growth performance and milk micronutrient analysis was analyzed in JMP 12 (Cary, NC) and LSMEANS Differences with Tukey-HSD Adjustment was used for all growth analysis. $P < 0.05$ was considered significant, non-significant factors were dropped and the model was rerun. When analyzing treatment effect, backfat loss (BF), weight loss, lactation feed intake, weaning weight (WW), and pre-weaning mortality were included in the model as response variables and treatment as the fixed effect and total number weaned as a covariate. A second statistical model was run to evaluate treatment effect on number born alive (NBA), number of stillborn (SB), total number born, (TNB), number weaned, pre-backfat (Pre-BF), and birth weight (BiW). Milk nutrients were analyzed for a treatment and day effect.

Data for microbiome analysis was analyzed through R package "stats" (version 2.15.3). Chimeras of the DNA were removed to prevent skewed results. An ASV table was generated through R, using Dada2 and a phylogenic tree in Motur in which the two were merged and a mapping profile self-created through excel for variables of interest were included. To analyze differential abundance of bacteria between sow v piglet, day and diet, DeSeq2 was used to calculate a negative binomial.

Results

Sow and Progeny Performance

Growth performance data are presented in Table 2. Treatment had no have an effect on the parity 4 sows lactation feed intake ($P = 0.0795$); however, a trend ($P < 0.10$) was observed where sows that were on the EXC diet consumed the most amount of feed (3.22 kg/d), while the REC sows consumed the least (2.83 kg/d). Furthermore, an effect of treatment on preweaning mortality was observed in which REC mortality was greater than EXC ($P = 0.037$). Treatment did not have an effect on WW ($P = 0 .190$) or NW ($P = 0.790$).

Treatment did not have an effect on birth weight ($P = 0.483$), Pre-BF ($P= 0.532$), or number of stillborn ($P = 0.342$), but did effect TNB ($P = 0.017$) in which CTL had the greatest (n = 17.5) and EXC had the least (n = 11.8), thus this data correlates directly to NBA ($P = 0.0189$) in which CTL had the greatest average number of piglets (n = 16.4) and EXC had the least (n = 11)($P = 0.249$).

Milk micronutrients

Micronutrient analysis is presented in Table 3. All nutrients analyzed had a time effect ($P < 0.05$) except for monounsaturated fat ($P = 0.147$), somatic cells ($P =$

0.154)and milk urea nitrogen (P = 0.284). Furthermore, short, medium and long chain fatty acids and saturated fatty acids, including C_{16} , C_{18}, and $C_{18\text{-}1}$ increased over time (P < 0.05). Diet had no effect on the milk analysis except for milk urea nitrogen (P = 0.011). Within the milk urea nitrogen analysis, it was found to be highest in the milk of sows on the EXC diet (60.68 mg/dL) and lowest in the REC (55.21 mg/dL) (P = 0.013).

Microbiome

Data was analyzed at the V_4 region of the 16s DNA sequence and first analyzed through an alpha refraction curve showing that all samples plateaued, indicating the reads were read to a sufficient level and analyzing a deeper region of the DNA was not necessary. All samples with less than 1000 reads were removed as were control.

Diet

To show the distribution of species taxonomy among dietary treatments, a Shannon Weiner diversity index and Simpson diversity index were created (Figure 1). As seen in Figure 1, there was similar taxonomy quantity and distribution among diet. Furthermore, in a principle component analysis there was no strong clustering by diet (Figure 2). In a figure showing different abundant taxa for piglets, the top 20 were recorded and showed Synergistes, Heliocobacter and Murdochiella to be the top 3 differentially abundant bacteria (Figure 3a). Among the top three, Synergistes and Heliocobacter were upregulated in the CTL group, while Murdochiella was upregulated in the EXC piglets (Figure 3b).

Piglet v Sow

To show the distribution of species taxonomy by day, Shannon Weiner diversity index and Simpson diversity index were created (Figure 4). As seen in Figure 4, the sows

displayed a more even distribution among its taxonomy when compared to the piglet. Furthermore, in a principle component analysis, there is a strong clustering, presumably causing d 1 piglets to cluster which is further discussed in the following section (Figure 5).

Day

In the principle component analysis, it was observed that d 80 and d 14 clustered more closely, whereas samples from d 1 clustered separately (Figure 6). In the analysis of the top 20 differentially abundant genus in sows based on day, among the top 20, Lactobacillus, Aerococcus, and Corynebacterium were the most differentially abundant (Figure 7a). Among these bacteria lactobacillus was upregulated on d 1 whereas Corynebacterium and Aerococcus were upregulated on d 14 (Figure 7b). No genus was upregulated in the sow on d 80 when compared to the other days (Figure 8a). For piglets, the top 20 differently abundant taxa were also analyzed, among the top 3 were Desulfovibrio, Christensenellaceae, and Alloprevotella. Among these top 3, all were upregulated on d 14 compared to d 1. Interestingly, of the top 20, only 1 genus, Sphingomonas, was upregulated on d 1 (Figure 8b). Among the top 20 most differentially abundant genus on 2 were seen in both the sows and piglets was analyzed by day. While Lactobacillus was present it both piglets and sows, it was upregulated in sows on d 1, but not upregulated in piglets until d 14. Lastly, both piglets and sows had Aerococcus upregulated of d 14.

Discussion

Sow and progeny performance

Results obtained from this study indicate that treatment did not have an effect on the birthweight or weaning weight of the piglets. However, it has been observed previously, that when a lactobacillus strain is fed directly to neonates there is an increase in body weight and feed efficiency, while also causing a decrease in diarrhea (Liu et al., 2014; Abe et al., 1995). In addition, strains of lactobacillus when fed to gestating sows have been seen to improve production performance, including increased litter weight and number of piglets weaned (Wang et al., 2014). While an increase in lactation feed intake was observed in the current study, the sows fed the lactobacillus fermentation product had decreased production performance. While total number born was shown to be affected by treatment, there seemed to be an issue in the farrowing of the sows on treatment because litter sizes were much smaller than usual for our farm. I believe it to be a coincidence that treatment effected total number born because after d 40 of gestation fetus are developed and will be mummified or stillborn if not viable (Flowers, 2017). Due to starting treatments of d 80 of gestation total number born should not have been affected. To look further into this data, number born alive and stillborn per sow was calculated and treatment did not have an effect. Treatment could have had an effect due to stillborns occurring during after d 100 of gestation (Flowers, 2017). Furthermore, supplementing a sow with lactobacillus has shown be strain specific on the effects it has on litter and reproductive performance (Food and Agriculture Organization World Health, 2002). Due to the strain of lactobacillus used in the study not being live, this may be the reason for altered results from those typically seen by probiotic supplementation with lactobacillus.

Milk Analysis

The milk in this study was analyzed by day and treatment. The only treatment effect observed on the milk composition was milk urea nitrogen. Milk urea nitrogen is typically used to measure protein metabolism (Hristov et al., 2018). Milk urea nitrogen is highly variable and reacts more to dietary changes than other components such as lactose (Jenkins and Mcguire, 2006). Milk urea nitrogen is not typically measured in sows; however, when high in dairy cows it is often associated with decreased reproductive performance (Broderick and Clayton, 1997). Milk urea nitrogen, when low, can indicate a protein deficiency, thus limiting milk output and milk protein yield. However, high milk urea nitrogen can be due to an excess of dietary protein or imbalance in the diet (Ishler, 2016). While milk urea nitrogen is not typically measured in sows. Plasma urea is and the two are closely related (Ishler, 2016). In a study by Rempel et al. (2018), it was observed that as plasma urea nitrogen increased in the sow, piglet average daily gain decreased. However, the increase in milk urea nitrogen in the EXC diet may be associated with greater lactation feed intake observed in the EXC diet. Mosnier et al. (2010) concluded that higher lactation feed intake resulted in greater plasma urea nitrogen. Studies suggest the reducing protein deamination, thus decreasing urea nitrogen from late gestation to end of lactation can result in increased piglet performance (Rempel et al., 2018).

A time effect for most of the milk nutrients was observed when compared from d 1 to d 14. Changes over time in milk composition were not surprising because as the piglets begin to grow, the milk nutrients change through lactation to meet their needs. As the piglet grows, the sows milk has balanced amounts of macro- and micronutrients to enhance growth and health in the piglet (Schutkowski et al., 2019). While some nutrients are affected by one factor more than another, it is important to feed a sow a balanced diet

in order for her milk to fit the needs of the growing piglet (Jenkins and McGuire, 2006). Milk fat is one nutrient that is easily affected my dietary intervention. Generally, lactose and protein within sows' milk are only affected by time and not diet (Hurley, 1997). In a study by Craig et al. (2019), they showed similar patterns of % lactose and % fat increasing from d 1 to d 14; however, they showed large decrease in % protein over time. Other studies looking at milk composition of sows have also found that short chain fatty acids are barely detectable through all stages of lactation and that most fatty acids present in sows' milk are palmitic acid, oleic acid, and linoleic acid (Csapo et al., 1996). Day of lactation greatly effects macronutrients, as seen in a study by O'Callaghan et al. (2020) where they also reported increases in saturated fats, however that was in bovine milk. Fatty acid content in milk greatly depends on lipid content in the diet and are important in the maturation of the piglet (Farmer and Quesnel, 2009). Typically, polyunsaturated fatty acids are high in colostrum, which was also observed in the current study, due to their effect on early brain development (Bai et al., 2017). Continuing on, we reported an increase of medium chain fatty acids, which agreed with other studies of medium chain fatty acids increasing lactation due to their ability to provide energy and improve feeding of the piglet (Zentek et al., 2011). Furthermore, medium chain fatty acids affect the microbiome of the piglet and inhibit harmful bacteria such as salmonella and coliforms while long chain fatty aid in inflammatory responses (Zentek et al., 2011; O'Connor-Robinson et al., 2014), similar trends of increased medium chain fatty acids were observed in the current experiment Milk composition is highly affected by time, diet, and species of the animal and understanding the effects each factor has on the nutrients can help enhance neonate performance.

Microbiome

The results of this study showed that diet did not affect alpha diversity; however, effects of day, and pig type (sow v piglet) did affect alpha diversity. In addition, beta diversity showed that diet, day, and pig type had an effect on the microbiome. Alpha diversity describes the individual sample's species richness and overall diversity, whereas beta diversity describes the differences in taxa when comparing one sample to another However, it seems that day plays the largest role in shaping the microbiome. While diet did not affect abundance of taxa and variation within sample it did cause an upregulation in specific bacteria per group. Gut microbiome is associated in aiding the hosts health, growth performance, and immune response and is easily altered at the beginning stages of life (Qin et al., 2010). Because newborn piglets just came from a sterile environment, it is common for piglets to have a microbiome that resembles that of the sow due to contact, nursing, and genetics (Bian et al., 2016).

Piglets go from a sterile unit within the womb to an extremely dense population of bacteria during birth to weaning resulting in an adult-like microbiome (Palmer et al., 2007). This is similar to the results seen in the current study, which showed as the piglets got older their microbiome clustered closer with that of the sows. Furthermore, a piglet's gut microbiome will vary much more than compared to that of the stable, consistent microbiome of the sow (Arnal et al., 2014). Studies show that when a sow is supplemented with lactobacillus as a probiotic, it may help maintain a healthy balance of beneficial gut bacteria and decrease harmful bacteria such as *E. Coli* and S. aureus (Wang et al., 2014). However, many studies have shown that there is no difference in gut microbiome when sows are fed a probiotic (Sarabria et al., 1997; Scharek et al., 2005;

Bohmer et al., 2006). Gedek (1993) explains that the small or no difference in the sow's microbiome is likely due to the already stable environment and altering adult microbiota is not easily done. However, in the current study it was shown that lactobacillus was upregulated on d 1 when compared to before starting the trial at d 80 of gestation and after being fed the supplement for 14 days. Interestingly, piglets showed an upregulation in lactobacillus on d 14, supporting the statement that as the pig ages its microbiome begins to resemble that of the sow.

While the sow microbiome may not alter, the microbiome of milk significantly alters from colostrum to late lactation, helping to develop the microbial colony of the piglet (Wei et al, 2018). The two most abundant bacteria genera are corynbacterium and streptococcus in colostrum, while other taxa including Lactobacillus, Ruminococcaceae, Lachnospiraceae, and Clostridiales are most abundant in late lactation (Wei et al., 2018). Similarly, piglets in this study showed an upregulation of lactobacillus, Ruminococcaceae, and Lachnospiraceae on d 14. Sow milk is an important part of developing the piglet microbiome during the first stages of life and to help reduce illness, therefore focusing directly on altering the piglet microbiome to increase health benefits is more beneficial than trying to alter it through sow diet.

Overall, for piglets to have an effect of a probiotic, it should be fed directly to the piglet, rather than supplementing the sow with it. Further research will need to be conducted to see if altering sow supplementation at higher levels has an indirect effect on piglet. While no positive correlations were seen in the experimental groups on the lactobacillus fermented product, research has shown the benefits of a sow's reproductive performance when given Lactobacillus

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Table 1. Ingredient composition and nutrient analysis of diets (as-fed basis) fed during gestation and lactation.

Item:	Gestation[1,2]	Lactation[3]
Ingredient, %		
Corn	77.25	65.68
Soybean Meal, 47.5 % CP	16.00	27.50
Tallow	3.00	3.00
Dicalcium Phosphate	1.90	2.33
Limestone	0.93	0.60
Salt	0.50	0.50
Vitamin Premix[4]	0.25	0.25
Trace Mineral	0.15	0.15
Phytase	0.02	-
Calculated composition		
ME (kcal/kg)	2605	2536
CP, %	11.74	15.75
Lys ana	0.56	0.85
Total P, %	0.67	0.80
Ca, %	0.87	0.90

[1]Gestation diet was fed from the day 1- 80 of breeding

[2]At d 81 of gestation sows continued on the gestation diet with the addition of Lactobacillus fermentation product. Sows allotted to REC diet, had their feed top dressed with 6g of Lactobacillus fermentation product, and sows allotted to EXC diet had their feed top dressed with 9g of Lactobacillus fermentation product. CTL sows had no Lactobacillus fermentation product added.

[3]Lactation diet was fed beginning at farrowing through d 21 post-farrowing, sows were put immediately back on gestation diet at d 21 post-farrowing.

[4]Provided per kilogram of diet for phase 3: 6,600 IU of Vitamin A, 600 IU of Vitamin D_3, 66 IU of Vitamin E, 4.40 IU of Vitamin K, 33.00 mg of Niacin, 22.05 mg of Pantothenic Acid, 11.00 mg of Riboflavin, and 22.05mg of Vitamin B_{12}, 550 mg of Choline Chloride, 1.65 mg of Folic Acid, 0.22 mg of Biotin

Table 2. Effects of feeding sows control (CTL; n = 10 sows n = 40 piglets), recommended (RESTR; n = 10 sows n = 40 piglets), or Excess (EXC; n = 10 sows n = 40 piglets) Lactobacillus fermentation product on sow and litter performance. Means in same row not connected by the same letter differ ($P < 0.05$).

	CTL	REC	EXC	*SEM*	*P*-Value
Pre-BF of sows, mm[1]	1.56[a]	1.60[a]	1.47[a]	0.092	0.532
Pre-weaning Mortality[2], %	0.27[a]	0.25[a]	0.10[b]	0.172	0.028
Progeny BiW, kg[3]	19.50[a]	18.60[a]	18.10[a]	1.221	0.483
Litter WW, kg[4]	75.00[a]	64.50[a]	70.10[a]	0.790	0.190
Lactation Feed Intake, kg	3.09[a]	2.83[a]	3.21[a]	3.912	0.079
Stillborn	1.10[a]	1.70[a]	0.80[a]	0.432	0.543
Number born alive	16.40[a]	15.70[a]	11.00[b]	1.366	0.018

[1]Gilt backfat at d 109 of gestation (pre-BF)
[2]proportion of (total number nursed - number weaned)/(number nursed) = preweaning mortality
[3] average total litter birth weight (BiW)
[4] average adjusted weaning weight (WW) of litter

Table 3. Effects of time on sow milk nutrient analysis.

Item	Day 1	Day 14	SEM	P Value
Fat %	6.63	8.21	0.183	< 0.0001
True Protein, %	8.86	11.38	0.274	< 0.0001
Lactose, %	4.20	5.50	0.054	< 0.0001
Casein B, %	7.46	3.64	0.232	< 0.0001
Free Fatty Acids, %	42.23	30.27	0.567	< 0.0001
Milk Urea Nitrogen, mg/dl	56.74	58.52	0.823	0.2843
Somatic Cells, scc/ml	3507.38	1193.00	802.45	0.154
C_{14}[1]	0.48	0.31	0.028	0.0038
C_{16}[1]	0.176	1.10	0.035	< 0.0001
C_{18}[1]	0.64	0.81	0.021	0.0002
$C_{18\text{-}1}$[1]	4.04	4.68	0.142	0.0280
Long Chain Fatty Acid[1]	4.28	5.30	0.177	0.0057
Medium Chain Fatty Acid[1]	0.563	1.30	0.062	< 0.0001
Short Chain Fatty Acids[1]	0.375	0.47	0.011	< 0.0001
Monounsaturated Fatty Acids[1]	4.12	4.50	0.130	0.147
Polyunsaturated Fatty Acids[1]	0.57	0.28	0.013	< 0.0001
Saturated Fatty Acids[1]	2.07	3.49	0.066	< 0.0001
Trans Fatty Acids[1]	0.365	0.02	0.018	< 0.0001

[1]Nutrients are measured in grams per 100 grams of milk

Figure 1. Alpha Diversity Plots of fecal microbiome samples of the sows and piglets based on Control (C), Excess (E), and Recommended (R) amounts of Lactobacillus fermentation product given during gestation.

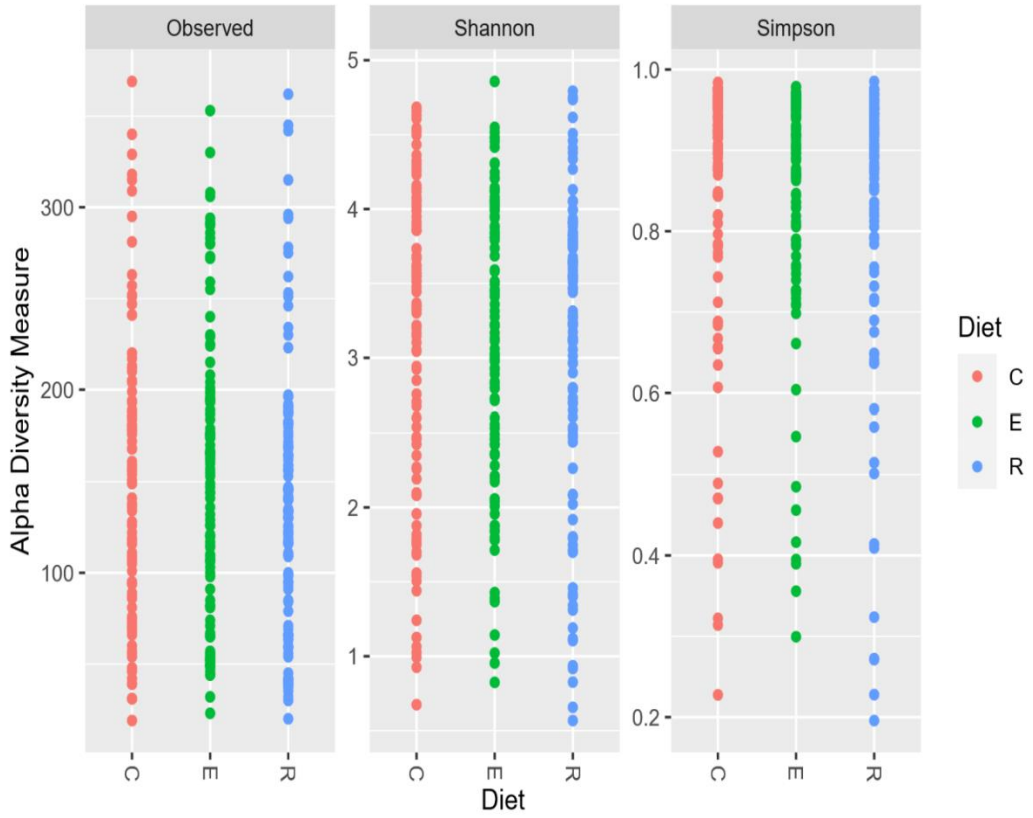

Figure 2. Principle Component Analysis of fecal microbiome samples of the sows and piglets based on Control (C), Excess (E), and Recommended (R) amounts of Lactobacillus fermentation product given during gestation.

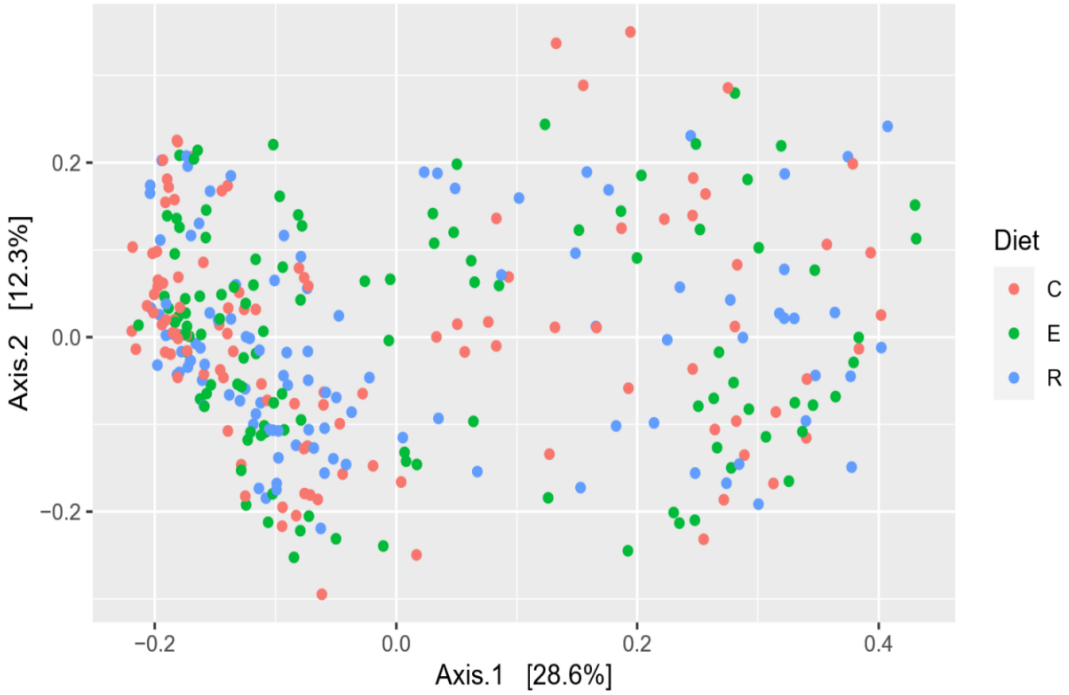

Figure 3a. Top 20 differentially abundant genus among piglets based on based on Control (C), Excess (E), and Recommended (R) amounts of Lactobacillus fermentation product given during gestation diet. Top numbers rank from 1 most differentially abundant to 20 least differentially abundant.

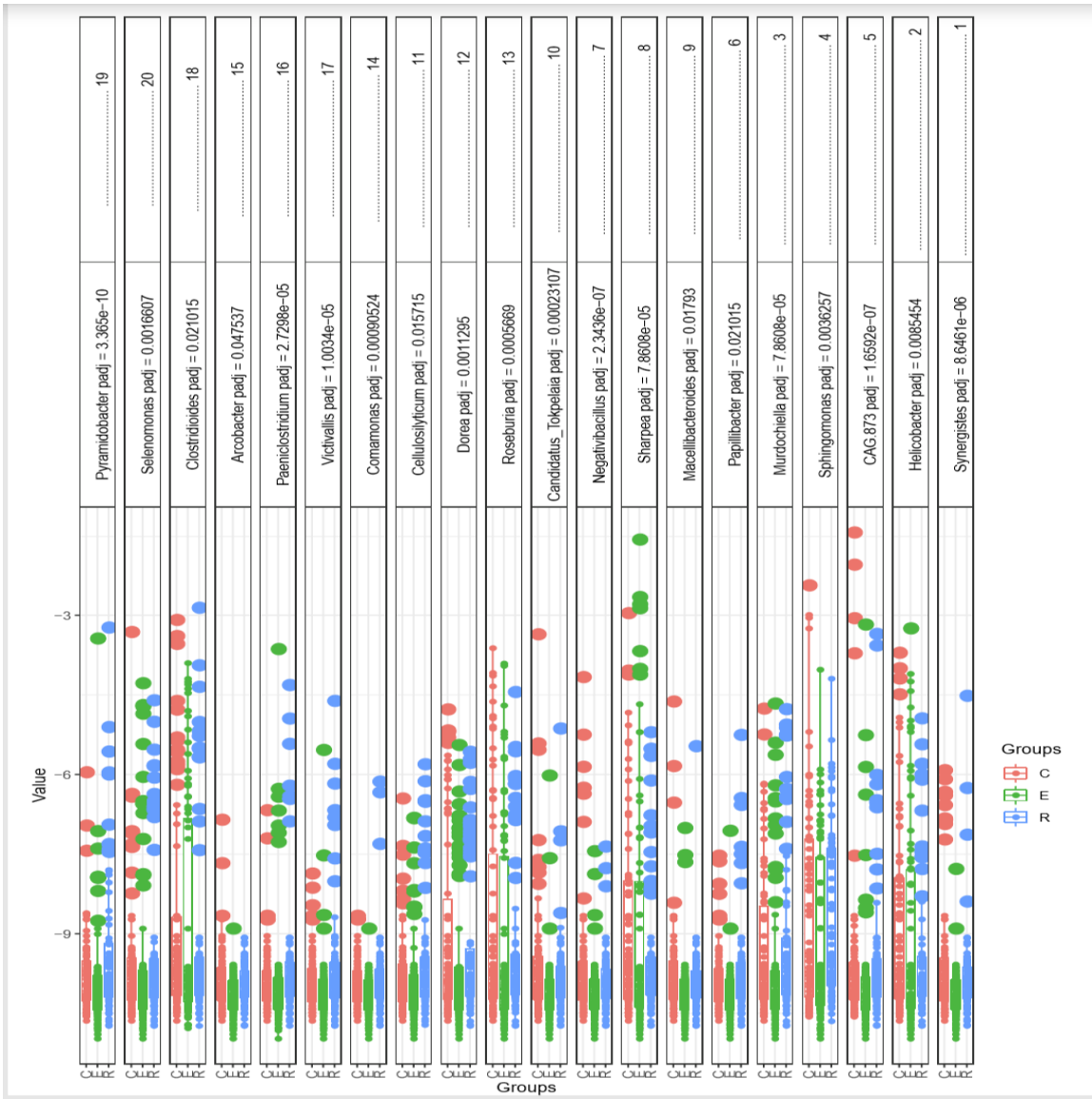

Figure 3b. The top 20 differentially abundant genus and which diet the genus is upregulated in.

Taxa	Base mean	Log2foldchain	Upregulated Diet
CAG.873	8.120309	-2.30685	CONTROL
Macellibacteroides	1.369074	-0.7344	CONTROL
Sphingomonas	14.84244	-1.21587	CONTROL
Synergistes	2.252637	-1.37362	CONTROL
Negativibacillus	2.16451	-1.63511	CONTROL
Sharpea	23.56659	-1.92748	CONTROL

Clostridioides	45.45186	-1.35921	CONTROL
Candidatus_Tokpelaia	1.964386	-1.15108	CONTROL
Arcobacter	1.211926	-0.59582	CONTROL
Helicobacter	21.04986	-1.32546	CONTROL
Dorea	9.789094	-1.38675	CONTROL
Roseburia	26.25564	-1.74803	CONTROL
Cellulosilyticum	2.383146	0.82345	EXCESS
Papillibacter	1.605313	0.720293	EXCESS
Victivallis	1.790265	1.28723	EXCESS
Pyramidobacter	3.372449	2.096059	EXCESS
Comamonas	1.335589	0.952906	EXCESS
Selenomonas	9.81473	1.415671	EXCESS
Paeniclostridium	3.479876	1.629275	EXCESS
Murdochiella	8.349789	1.663336	EXCESS

Figure 4. Alpha Diversity Plots of fecal microbiome samples of the sows and the piglets.

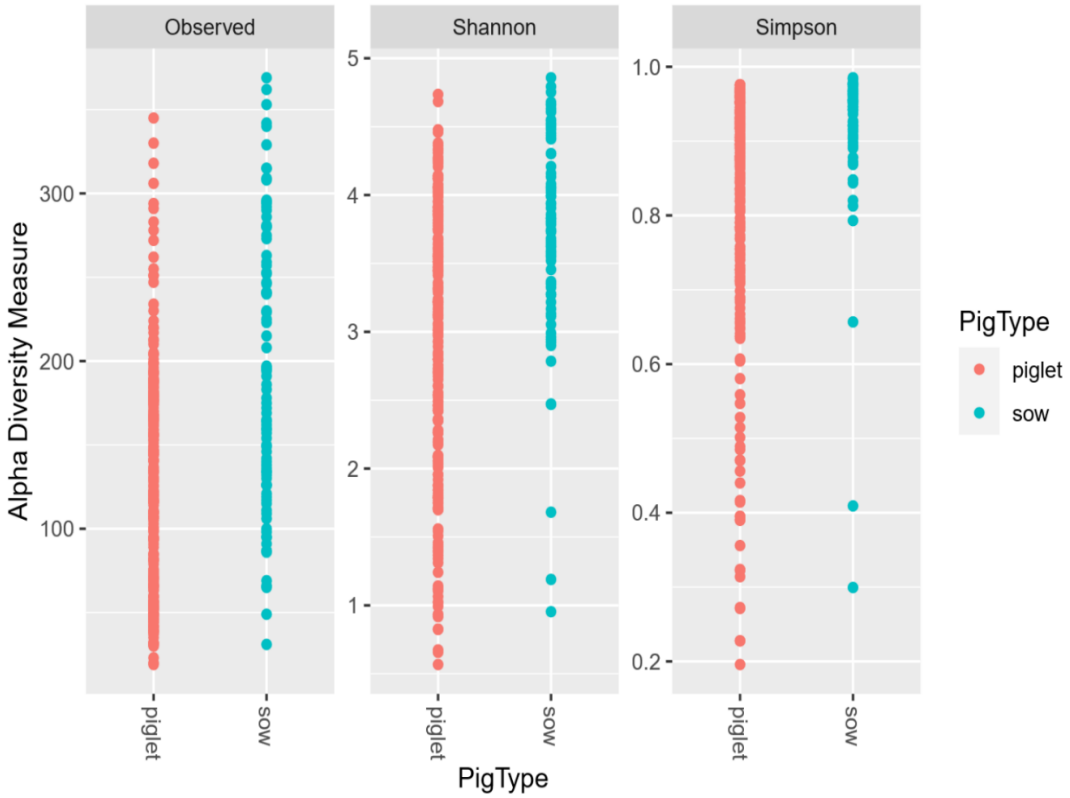

Figure 5. Principle Component Analysis of fecal microbiome samples of the sows and the piglets.

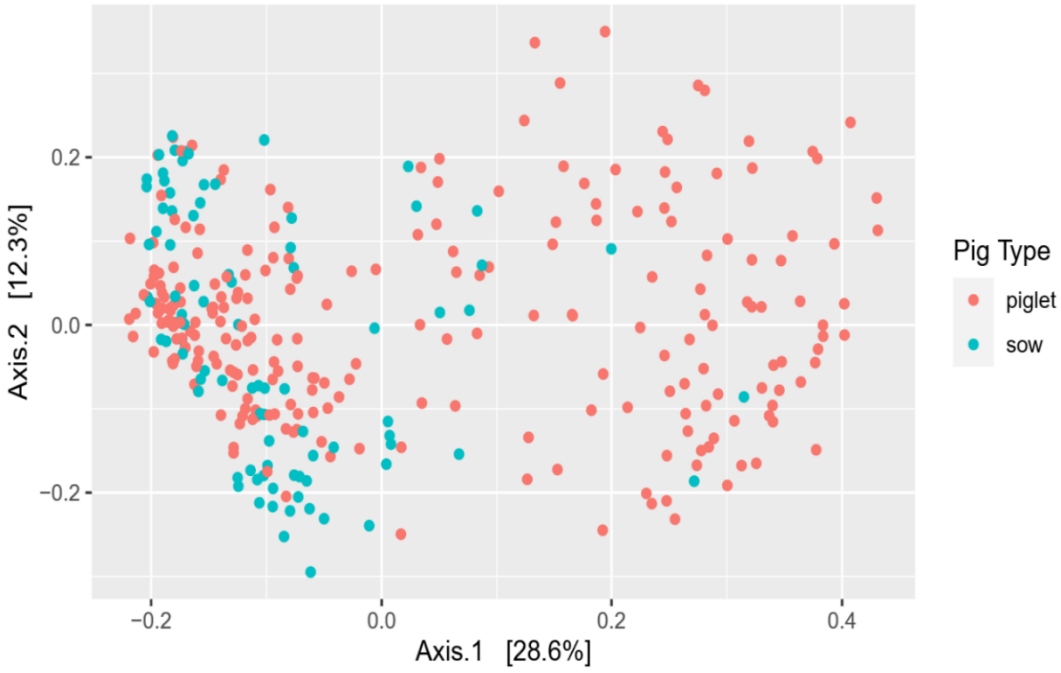

Figure 6. Principle Component Analysis of fecal microbiome samples sows based on d 80 of gestation, and piglets and sows d1 and d 14 post-farrowing

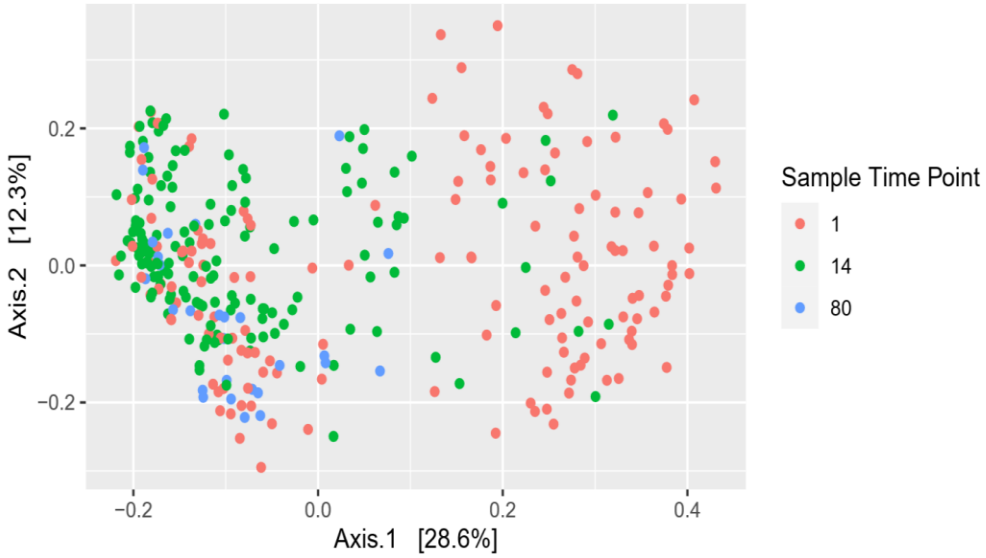

Figure 7a. Top 20 differentially abundant genus among sows based on day. Top numbers rank from 1 most differentially abundant to 20 least differentially abundant. 1 and 14 represent days post-farrowing, 80 represents day of gestation.

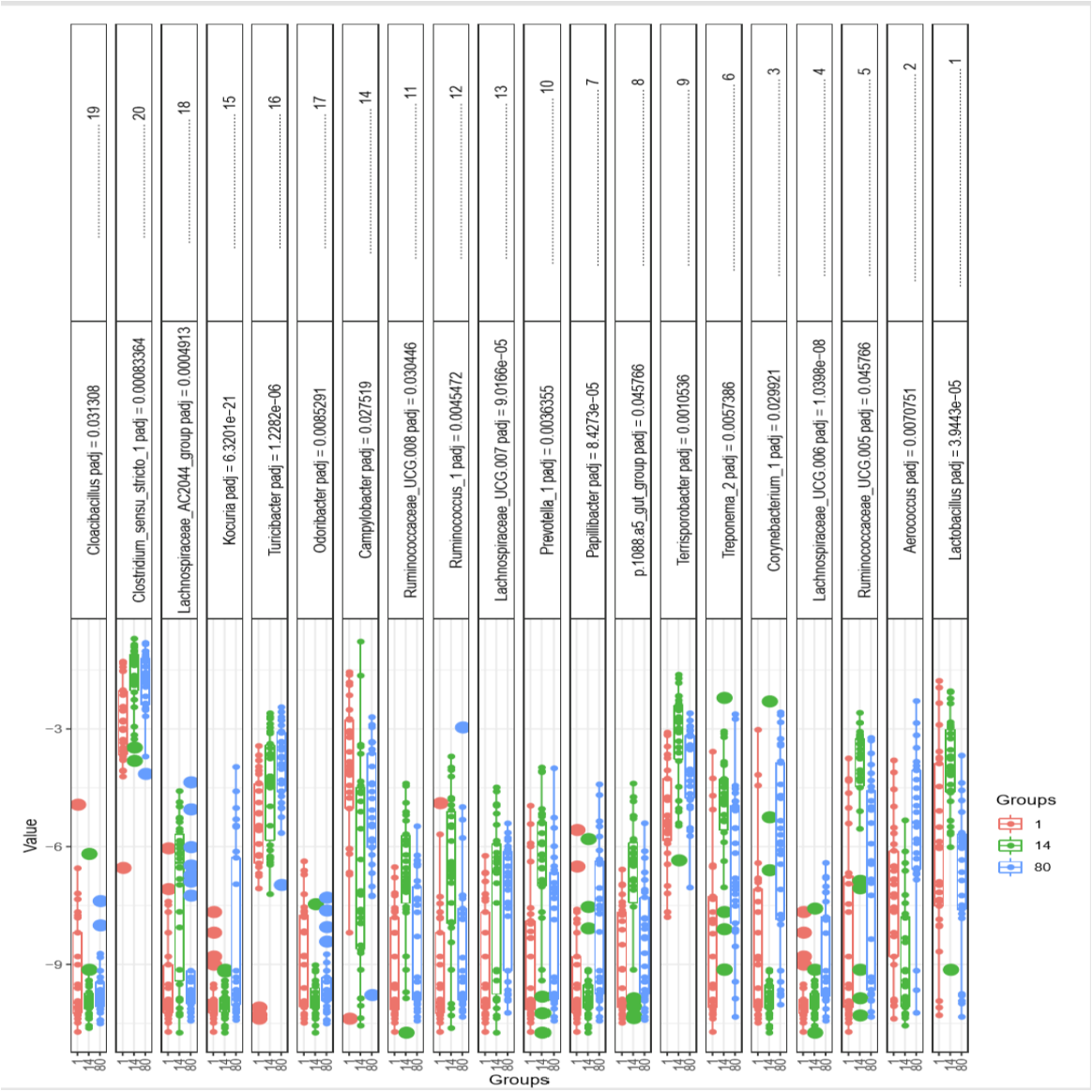

Figure 7b. The top 20 differentially abundant genus in sows and which day the genus is upregulated.

Genus	baseMean	log2FoldChange	Upregulated diet
Odoribacter	3.268466	-1.28286	1
Cloacibacillus	2.177704	-1.04729	1
Lactobacillus	409.9804	-2.56958	1
Campylobacter	708.0293	-1.71323	1
Ruminococcaceae_UCG.005	260.8987	1.56304	14
Ruminococcaceae_UCG.008	27.79698	1.350375	14
Papillibacter	14.47557	2.539898	14
Treponema_2	173.7138	1.955315	14
p.1088.a5_gut_group	23.3093	1.192525	14
Ruminococcus_1	51.13822	2.157821	14
Kocuria	18.33736	5.689515	14
Corynebacterium_1	163.2831	2.108417	14
Clostridium_sensu_stricto_1	4048.245	1.293897	14
Turicibacter	471.3968	2.356009	14
Aerococcus	117.9809	1.887391	14
Terrisporobacter	803.7693	1.517556	14
Prevotella_1	39.35402	1.848552	14
Lachnospiraceae_UCG.006	3.512297	2.920941	14
Lachnospiraceae_UCG.007	31.85309	2.391977	14
Lachnospiraceae_AC2044_group	24.78305	2.34646	14

Figure 8a. Top 20 differentially abundant genus among piglets based on day 1 and 14. Top numbers rank from 1 most differentially abundant to 20 least differentially abundant.

Figure 8b. The top 20 differentially abundant genus among piglet and which day the genus is upregulated

Genus	baseMean	log2FoldChange	Day Upregulated
Sphingomonas	17.77401	-1.2861	1
Ruminococcaceae_UCG.002	640.6805	2.463065	14
dgA.11_gut_group	20.3191	0.848468	14
Desulfovibrio	134.0259	2.166911	14
Methanosphaera	2.013986	0.927083	14
Akkermansia	117.1719	2.782081	14
Kingella	1.868282	1.000502	14

Ruminococcaceae_UCG.010	28.49456	2.455045	14
Fournierella	77.36483	3.508941	14
UBA1819	70.13221	2.344959	14
Hydrogenoanaerobacterium	14.25478	2.964571	14
Christensenella	2.63739	1.198237	14
Actinomyces	168.8015	2.61883	14
Eubacterium	22.17031	2.223185	14
Aerococcus	11.12528	1.682152	14
Lactobacillus	610.2788	0.885428	14
Alloprevotella	82.55317	1.696141	14
Phascolarctobacterium	175.5552	1.908747	14
Bacteroides	2481.072	1.173889	14
Lachnoclostridium	1250.962	1.74126	14

Appendix 1.

Survey for: Impacts of Participation in Undergraduate Research on Students Majoring in Animal Science

Please take the survey based on your experience in one department, the survey may be taken multiple times if a student has worked for multiple departments.

1. Please indicate your sex

- Male
- Female
- Prefer Not to Answer

2. What is your age?

- 18-19
- 20-21
- 22-23
- 24+

3. What year in school are you?

- Freshman
- Sophomore
- Junior
- Senior
- Senior+

* 4. During your time as an undergraduate research student, How do you pay for your school tuition? (Check all that apply)

- Myself
- Student Loans

- Parents/Relatives
- Scholarship
- Other _____

* 5. Please specify your ethnicity origin

- White
- Pacific Islander or Native Hawaiian
- Black or African American
- Native American or American Indian
- Asian
- Other
- Other (please specify)

* 6. Are you a Nebraska resident?

- Yes
- No

* 7. Are you a United States citizen? (If "Yes" question 8 will be skipped automatically)

- Yes
- No

* 8. What is your home country?

* 9. Is English your first language? (If "Yes" question 10 will be skipped automatically)

- Yes
- No

* 10. What is your first language?

* 11. Which reference best describes the population of your hometown in which you spent a majority of your childhood prior to college?

- ○ <1,000
- ○ 1,000-5,000
- ○ 5,001-10,000
- ○ >10,000

* 12. How did you hear about the undergraduate research job? (Check all that apply)

- ☐ A professor
- ☐ A friend
- ☐ The Animal Science Website
- ☐ An Email
- ☐ Other (please specify)

* 13. What is the focus of your degree in Animal Science? (Mark all that apply)

- ☐ Animal Biology & Biotechnology
- ☐ Food Animal Production & Management
- ☐ Business & Communications
- ☐ Companion Animal Science
- ☐ Equine Science
- ☐ Meat Science
- ☐ Veterinary Animal Science
- ☐ Not an Animal Science Major
- ☐ Other (please specify)

* 14. What is the highest level of school your complete by your father?

* 15. What is the highest level of school completed by your mother?

* 16. Do you plan to continue on to graduate school?

- Yes
- No
- Unsure

* 17. Will you be attending graduate or professional school in the academic year immediately following graduation?

- Yes
- No
- Unsure
- Not Applicable

* 18. Have you been accepted into graduate school?

- Yes
- No
- Not Applicable

* 19. Do you plan to attend Graduate School at University of Nebraska - Lincoln?

- Yes
- No
- Unsure
- Not Applicable

* 20. Please select ONE area of research you worked with. Answer questions 21-37 according to this answer, (the survey may be taken multiple times should you have worked in numerous departments)

- Ruminant Nutrition - Beef

- Ruminant Nutrition - Dairy
- Non-Ruminant Nutrition - Swine
- Non-Ruminant Nutrition - Poultry
- Physiology
- Breeding and Genetics
- Meat Science
- Other (please specify)

* 21. What was the average number of hours you worked a week?

- 1-5
- 6-10
- 11-15
- 16-20
- >20

* 22. How many total semesters have you worked in undergraduate Animal Science research, in which you helped professors/students with research? (each summer counts as 1 semester) (If you are currently working as an undergraduate research student count this semester as 1)

- 1-2
- 3-5
- 6-8
- 9+

* 23. Of the following, which had a greater positive impact on your undergraduate research experience?

- Graduate Students
- Professors

None

* 24. Please rate the following statements based on the phrase below.

My time spent in undergraduate research...

	Not at All	Slightly	Somewhat	Moderately	Extremely	Not Applicable
Has helped me in classes						
Has changed my feelings in a positive way about grad school						
Has prepared me for graduate school						
Relates to my future education goals						

* 25. Please rate the following statements based on the phrase below:

During undergraduate research....

	Never	Occasionally	Sometimes	Frequently	Always	Not Applicable
I felt comfortable asking questions when I was unsure about things						
I felt comfortable performing tasks on my own that						

	Never	Occasionally	Sometimes	Frequently	Always	Not Applicable
were related to the lab's research						
I received adequate training before working with research animals						
I received adequate training before working in the lab with chemicals						

* 26. Please rate the following statements based on the phrase below:

During undergraduate research....

	Never	Occasionally	Sometimes	Frequently	Always	Not Applicable
The research I participated in is what I expected it to be						
I applied concepts I learned in previous classes, while working on research						
I enjoyed working with the head faculty of the lab group						
I enjoyed working with the graduate						

	Never	Occasionally	Sometimes	Frequently	Always	Not Applicable
students in the department during research						

* 27. Please rate the following statements based on the phrase below.

My time spent in undergraduate research...

	Not at All	Slightly	Somewhat	Moderately	Extremely	Not Applicable
Has given me a better appreciation for animal research						
Changed my academic path for graduate school						
Made me go outside my comfort zone						
Was good use of my time						

* 28. Please rate the following statements based on the phrase below.

During your time as an undergraduate research student how would you rate...

	Poor	Fair	Neutral	Good	Excellent	Not Applicable
Your relationship with the faculty during undergraduate research						

Your relationship with the graduate during undergraduate research						
The availability of the faculty within the lab you worked in for when you had questions						
The availability of the graduate students within the lab you worked in for when you had questions						

* 29. Please rate the following statements based on the phrase below.

I will...

	Never	Not Likely	Possibly	Very Likely	Definitely	Not Applicable
Recommend this lab group to other students						
Recommend University of Nebraska - Lincoln to						

incoming freshmen						
Use what I learned in undergraduate research in the future						
Continue to work as an undergraduate research student						

* 30. Please rate the following statements based on the phrase below.

During your time as an undergraduate research student how would you rate...

	Poor	Fair	Neutral	Good	Excellent	Not Applicable
Your understanding of the research conducted						
The condition of the lab equipment						
The condition of the research animals						
The attitude of the students you worked with						
The attitudes of the faculty						

members you worked with						

* 31. Please rate the following statements based on the phrase below.

Undergraduate research has influenced the following statements in a positive manner

	Not at all	Slightly	Somewhat	Very	Extremely	Not Applicable
Listening skills						
Microsoft Word skills						
PowerPoint skills						
Excel skills						
Statistical program skills						

* 32. Please rate the following statements based on the phrase below.

I will...

	Never	Not Likely	Possibly	Very Likely	Definitely	Not Applicable
Seek advice from the faculty members of this lab for future academic plans						
Seek advice from the faculty members of this lab for future career plans						
Seek advice from the graduate students of this lab for						

future academic plans						
Seek advice from the graduate students of this lab for future career plans						

* 33. Please rate the following statements based on the phrase below.

Undergraduate research has influenced the following statements in a positive manner

	Not at all	Slightly	Somewhat	Very	Extremely	Not Applicable
Critical thinking						
How to support my hypothesis with research						
Application of the scientific method						
Perception of animal research						
Interest in a career with research						
Communication skills						

* 34. Please rate the following statements based on the phrase below

I feel that...

	Strongly Disagree	Disagree	Neutral	Agree	Strongly Agree	Not Applicable
Graduate students within the lab group were eager to teach me						
Faculty within the lab group were eager to teach me						
Graduate students within the lab group were gracious off my help						
Faculty within the lab group were gracious off my help						

* 35. Please rate the following statements based on the phrase below

I feel that...

	Strongly Disagree	Disagree	Neutral	Agree	Strongly Agree	Not Applicable
My time was well spent in undergraduate research						
I was eager to work in undergraduate research						
Graduate students within the lab group were						

	Strongly Disagree	Disagree	Neutral	Agree	Strongly Agree	Not Applicable
respectful to me						
Faculty within the lab group were respectful to me						

* 36. What would you change about your experience working as an undergraduate research student?

_____

* 37. Should you have any further comments on the topic please leave a comment below.

_____

Appendix 2.

Survey for: Assessment of undergraduate student learning in an Animal Science major

Please indicate your response for each question on the Scantron sheet. No name is required. Thank you for giving your honest answers and contributing to the improvement of the Animal Science major.

Understanding

Presently, I understand the following concepts:

1.	Biology and chemistry of the life sciences and application of the principles to animal nutrition, growth, reproduction, genetics and management of animals and their products	Not at all A	Just a little B	Somew hat C	A lot D	A great deal E
2.	How to develop animal nutrition, growth, reproduction, genetics and management recommendations related to the specific animal or animal product in the career paths related to my selected option	Not at all A	Just a little B	Somew hat C	A lot D	A great deal E
3.	The terms, facts and concepts of Animal Science	Not at all A	Just a little B	Somew hat C	A lot D	A great deal E
4.	How ideas we explore in Animal Science classes relate to ideas I have encountered in other classes.	Not at all A	Just a little B	Somew hat C	A lot D	A great deal E
5.	How ideas we explore in my biology and chemistry classes relate to my Animal Science classes	Not at all A	Just a little B	Somew hat C	A lot D	A great deal E
6.	How studying Animal Science helps people address real-world issues	Not at all A	Just a little B	Somew hat C	A lot D	A great deal E

Skills

Presently, I can:

7.	Critically read articles about issues raised in Animal Science classes	Not at all A	Just a little B	Somewhat C	A lot D	A great deal E
8.	Recognize a sound argument and appropriate use of evidence	Not at all A	Just a little B	Somewhat C	A lot D	A great deal E
9.	Develop a logical argument	Not at all A	Just a little B	Somewhat C	A lot D	A great deal E
10.	Write documents in discipline-appropriate style and format	Not at all A	Just a little B	Somewhat C	A lot D	A great deal E
11.	Work effectively with others	Not at all A	Just a little B	Somewhat C	A lot D	A great deal E
12.	Prepare and give oral presentations	Not at all A	Just a little B	Somewhat C	A lot D	A great deal E

Attitudes

Presently, I am:

13.	Enthusiastic about Animal Science	Not at all A	Just a little B	Somewhat C	A lot D	A great deal E
14.	Confident that I can be successful in an Animal Science career	Not at all A	Just a little B	Somewhat C	A lot D	A great deal E

15.	Comfortable working with complex ideas	Not at all A	Just a little B	Somewhat C	A lot D	A great deal E
16.	Confident in my ability to understand societal and ethical issues related to animals	Not at all A	Just a little B	Somewhat C	A lot D	A great deal E
17.	Willing to seek help from others (teacher, peers, TA) when working on an academic problem	Not at all A	Just a little B	Somewhat C	A lot D	A great deal E
18.	Prepare and give oral presentations	Not at all A	Just a little B	Somewhat C	A lot D	A great deal E

Integration of learning

Presently, I am in the habit of:

19.	Applying principles of Animal Science to new problems and situations	Not at all A	Just a little B	Somewhat C	A lot D	A great deal E
20.	Using systematic reasoning in my approach to problems	Not at all A	Just a little B	Somewhat C	A lot D	A great deal E

Knowledge of the Animal Sciences

21. Knowing that the B allele that codes for Black is dominant to the b allele that codes for red, what are the allelic and phenotypic frequencies if 500 animals are BB, 400 are Bb, and 100 are bb?
    A. Frequency of B is 75%, frequency of b is 25%, frequency of black hided animals is 75% and frequency of red hided animals is 25%.
    B. Frequency of B is 70%, frequency of b is 30%, frequency of black hided animals is 50% and frequency of red hided animals is 50%.
    C. Frequency of B is 70%, frequency of b is 30%, frequency of black hided animals is 90% and frequency of red hided animals is 10%.

D. Frequency of B is 90%, frequency of b is 10%, frequency of black hided animals is 90% and frequency of red hided animals is 10%.

22. A Breeding Value can be described as
    A. The sum of the average effect of each gene.
    B. Half of the sum of the average effect of each gene.
    C. A value between 0 and 1 related to the superiority of parents.
    D. An animal's own performance divided by the average performance of a group of similarly managed animals.

23. Heritability is defined as
    A. The ratio of additive genetic variance over phenotypic variance.
    B. The proportion of phenotypic differences observed that are due to differences in additive genetic effects.
    C. The probability that offspring will receive a given trait from their parents.
    D. Both A and B.
    E. Both B and C.

24. Genetic gain per year will be increased if
    A. The average age of parents becomes greater and accuracy of selection stays the same.
    B. A larger proportion of selection candidates are retained.
    C. The amount of genetic variation is decreased.
    D. The average age of parents decreases.

25. Heterosis will have the largest impact on which trait below?

    A. Height ($h^2 = 0.7$)
    B. Average daily gain ($h^2 = 0.4$)
    C. First service conception ($h^2 = 0.1$)
    D. Age at puberty ($h^2 = 0.4$)

26. The omasum is located just caudally to the
    A. Pyloric sphincter
    B. Ileum
    C. Reticulo-rumen complex
    D. Cecum

Water is generally considered:

    A. hydrophobic
    B. polar
    C. a compound with the formula H_2O_2
    D. A and B

28. The essential or indispensable amino acids:

A. are more important physiologically than the nonessential or dispensable amino acids.
B. include the amino acids alanine, glutamate, and asparagine
C. must be included in total or in part in the diet
D. can all be synthesized from water and glucose

29. Cell wall components are very important to maintaining plant structure and function. Which of the following accurately describes the cell wall components of forages and grains?

A. Neutral detergent fiber (NDF) that includes: cellulose and lignin only
B. Neutral detergent fiber (NDF) that includes: cellulose, hemicellulose, and lignin
C. Acid insoluble ash that includes oligosaccharides and lignin
D. Acid detergent fiber (ADF) that includes: cellulose, hemicellulose, and lignin

30. The primary difference between a saturated and unsaturated fatty acid is:

A. the number of carbons in the fatty acid
B. saturated fatty acids are only found in ruminant products
C. unsaturated fatty acids cannot be used in milk replacers
D. saturated fatty acids do not contain any double bonds between carbons in the fatty acid chain.

31. Which of the following bacteria is a major food safety issue for the beef industry?

A. Streptococcus pyogenes
B. Escherichia coli O157:H7
C. Clostridium botulinum
D. Listeria monocytogines

32. Which ion is responsible for the color of meat products?

A. Fe
B. Ca
C. Na
D. Cu

33. From which muscle do we cut the T-bone steak and the New York strip steak?

A. Longissimus
B. Biceps femoris
C. Triceps brachii
D. Semimembranosus

34. Which fat depot has the biggest impact on the flavor and juiciness of meat products?
    A. Intramuscular fat
    B. Visceral fat
    C. Subcutaneous fat
    D. Intermuscular fat

35. There are three primary types of growth, which type of growth is defines as an increase in the cell size
    A. Accretion
    B. Hypertrophy
    C. Hyperplasia

36. Where does spermatogenesis occur?

    A. Seminiferous tubules
    B. Corpus spongiosoma
    C. Prostate gland
    D. Scrotum

37. The filtration process of urine formation occurs in the _____.

    A. Distal convoluted tubule
    B. Glomerulus
    C. Renal pelvis
    D. Proximal convoluted tubule

38. The mare has one location on her ovary where ovulation takes place. What is the structure called?

    A. Ovulation central
    B. Ovulation fornix
    C. Ovulation fossa
    D. Ovulation orifice

39. The axial skeleton is composed of the:

    A. skull, vertebrae, ribs, and sternum.
    B. skull and vertebrae.
    C. ribs, sternum, and pectoral and pelvic girdles.
    D. skull, vertebrae, and pectoral and pelvic girdles

40. When the right atria contracts, blood goes through the _____.

A. tricuspid valve
B. cranial vena cava
C. pulmonary valve
D. aortic valve

41. Major

What best characterizes your major in college (pick only one):

A.	Major in Animal Science
B.	Not a major in Animal Science
C.	Undecided at this time
D.	Plan on becoming a major in Animal Science
E.	Plan on becoming a major in another area

42. GPA

What is your current GPA?	4.00-3.60 A	3.01-3.59 B	2.51-3.00 C	2.01-2.5 D	2.00 or lower E

Appendix 3.

Survey for: Veterinary Student Case Study Project Leads to Development of Professional Skills

Gender

M F

Year In Vet School

1 2 3 4

Ethnicity

Hispanic	African-american
multiracial	foreign National
Asian americ.	Caucasian
Other	native American

Are you a vet student or Graduate Student

Vet Graduate

Rate 1-5; 5 being yes very much; 1 being no not at all

I was given adequate instruction for the project

1 2 3 4 5

I understood what was expected out of me for this project

1 2 3 4 5

The teacher was easy to reach for questions and further instruction

1 2 3 4 5

Getting the audience involved, helped further your thinking on your case

1 2 3 4 5

I was able to use previous knowledge to connect it to new concepts

1 2 3 4 5

I enjoyed working on this project

1 2 3 4 5

I feel that PowerPoint was the best way to present my case study

1 2 3 4 5

This project enhanced my understanding of nutrition and metabolic disorders

1 2 3 4 5

My classmates were willing to actively participate in the project during question time

1 2 3 4 5

How many hours did you work on this project outside of class

After completing this project I have a better appreciation for nutrition

1 2 3 4 5

I would like to take more nutrition classes

1 2 3 4 5

I have a better understanding of how to present research

1 2 3 4 5

This project helped better my professional career

1 2 3 4 5

I gained experience using a systematic approach to animal assessment,

dietary assessment, and evaluation of feeding-management practices in several case-based problems or simulated events

1 2 3 4 5

enjoyed working with your professor

1 2 3 4 5

I enjoyed working in a group

1 2 3 4 5

All group members participate equally

1 2 3 4 5

After Completing this project, I have a better appreciation for animal research

1 2 3 4 5

I applied concepts I learned in previous classes, when working on this project

1 2 3 4 5

Completing this project improved my critical thinking

1 2 3 4 5

I had to improve my communication skills to work on a group project

1 2 3 4 5

I better understand how to support my ideas with research

1 2 3 4 5

Completing this project relates to my future education goals

1 2 3 4 5

I have a better understanding of the application of the scientific method

1 2 3 4 5

I am more interested in a career with research after completing this case study

1 2 3 4 5

What would you change about your undergraduate research time

_____

What did you like about the project

_____

Do you feel that students or professor had a greater impact on your learning in this class

Students Professors