The Impact of Feed Additives and Calcium Concentration and Source on Broiler Performance, Nutrient Utilization, and Intestinal Health Under Different Disease States

by

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A dissertation submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Doctor of Philosophy

Auburn, Alabama
May 10, 2025

Keywords: broiler, phytase, calcium butyrate, bacitracin methylene disalicylate,
limestone particle size, calcium concentration

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ABSTRACT

Optimizing broiler nutrition is essential for maintaining performance, enhancing nutrient utilization, and supporting intestinal health, especially when birds face various disease challenges. The reduction or removal of antibiotic growth promoters (**AGP**) in broilers has presented additional challenges for nutritionists, necessitating the development of alternative feeding strategies that sustain broiler performance and health while minimizing feed costs. These feeding strategies should aim to replicate performance outcomes previously achieved with AGP, though this remains difficult due to the complex mode of action of AGP. The present research explored two nutritional strategies to address AGP removal in broiler feeding: (1) feed additives and (2) diet manipulation. Feed additives like phytase and butyrate may help to alleviate the negative effects of AGP removal on broiler growth. Phytase, an exogenous enzyme, is commonly included in broiler diets to degrade phytate and enhance phosphorus (**P**) digestibility. Butyrate, an organic acid, improves intestinal health by modulating the microbiota and providing energy to intestinal epithelia. To evaluate the first nutritional strategy, broilers were provided with 1 of 9 treatments that included varying levels of phytase, calcium butyrate (**CB**), and bacitracin methylene disalicylate (**BMD**). Supplementing BMD increased early broiler body weight (**BW**), particularly when combined with 1,500 FTU/kg of phytase, but not with 500 FTU/kg. Depending on phytase inclusion, broilers fed a diet with CB exhibited similar BW compared to those fed BMD. Additionally, phytase at 1,500 FTU/kg enhanced fat and P digestibility, and apparent ileal digestible energy (**AIDE**). Intestinal physiology and cecal microbiota composition were influenced by CB and BMD, with changes in villus height, tight-junction gene expression, and cecal microbial evenness, depending on dietary phytase concentrations. These findings highlight the complex interactions between feed additives and their potential to modulate broiler performance and intestinal health. The second nutritional strategy involved manipulating dietary components, such as calcium concentration and limestone particle size, to help mitigate performance and mortality impacts from enteric diseases. Broiler diets are often unintentionally formulated with excess calcium, which may exacerbate necrotic enteritis by supporting NetB and α-toxin activity, leading to reduced nutrient utilization and compromising broiler health. Excess calcium can also increase calcium-phytate formation, resulting in undigested nutrients that become available to intestinal microbiota, potentially promoting proliferation of pathogenic bacteria. To evaluate this strategy, broilers were assigned to 1 of 7 treatments, including an unchallenged control and 6 enterically challenged groups fed diets with varying limestone particle sizes and calcium concentrations. The enteric challenge reduced broiler performance without affecting mortality. Reducing dietary calcium by 0.10 percentage units from recommended levels maintained broiler performance, tibia mineralization, and AIDE during an enteric challenge. Furthermore, calcium and P digestibility increased when dietary calcium was reduced. However, feed conversion and nutrient utilization responses varied depending on limestone particle size. Understanding the interaction between dietary calcium and limestone particle size could help to mitigate the impact of enteric diseases. Ultimately, to develop sustainable solutions that enhance broiler growth and health, broiler nutritionists must continue exploring AGP alternatives and further understand interactions between feed additives and dietary components.

DEDICATION

The author would like to dedicate this dissertation to his parents, Salvatore and Missie Gulizia, for their endless support, love, and encouragement throughout his life. Their continuous sacrifices and unwavering belief have shaped him into the person he is today. Thank you, Dad and Mom, for your unconditional love and for always believing in me. I am forever grateful.

ACKNOWLEDGMENTS

The author wishes to extend sincere appreciation to Dr. Wilmer Pacheco for his mentorship and guidance throughout this journey. Dr. Pacheco provided the opportunity to pursue both an M.S. and Ph.D. in Poultry Science, and over the past five years, his support has profoundly shaped the author's educational experience at Auburn University. His ability to foster a family environment in the lab made graduate student life more manageable, and the author will always cherish the lab cookouts and nights spent in downtown Auburn.

Additional thanks are extended to Drs. Allen Davis, Ruediger Hauck, Samuel Rochell, and Kelsy Robinson for their service as committee members and to Dr. Marko Rudar for serving as the University Reader. The author would also like to express gratitude to Dr. Klint McCafferty for his support of these research projects.

These projects would not have been possible without the assistance of the Auburn University Charles C. Miller Jr. Poultry Research Center and Poultry and Animal Nutrition Center staff. Their extensive applied knowledge was key in the author's success during feed manufacturing and experimental trials. The author is also grateful for the invaluable support from other faculty, staff, and graduate and undergraduate students from the Department of Poultry Science.

The author appreciates the endless support during his Ph.D. program from Dr. Pacheco's lab group: Isaac Vargas, Rodrigo Hernandez, Susan Bonilla, Eva Guzman, Urias Castillo, Leticia Orellana, Leopoldo Almeida, Patricia Soster, Sureerat Thuekeaw, Cristina Simões, Erasmo Flores, Isadora Pallaoro, Beatriz Onishi, Maria Jose Brizuela, Jibely Martínez, Fabio Valenzuela, Esteban Rosero, Isabella Dias, Aaron Galvarro, and

Pablo Barrera. Their friendship, encouragement, and dedication helped push the author forward, especially on the long and exhausting days.

A special thank you is extended to Dr. Kevin Downs. It cannot be overstated that the author would not be in this position without his unwavering support. More than a mentor, Dr. Downs has been a true friend, standing by the author through the highs and lows of this academic journey. There are few moments in life when you meet someone who leaves a lasting, positive impact, influencing both your professional path and personal growth. Without a doubt, Dr. Downs has been instrumental in the author’s success, and for that, the author will always be grateful. War Eagle forever, my friend!

Heartfelt gratitude is also extended to close friends, Jason and Casey Steele. They have continued to provide support and create countless moments of laughter and fun. The days throughout this journey were often long and challenging, but their friendship brought much needed joy and balance. The author is deeply grateful for their kindness, generosity, and the many ways they contributed to this journey. Also, special thanks are owed to Demi Menotti and her family for making Auburn, AL and Starkville, MS feel like home.

Lastly, the author is grateful to his wife, Anna K. Gulizia, for her never-ending support. This dissertation is the culmination of the hard work they both have put in over the past few years. Throughout this academic journey, her optimism and encouragement helped the author navigate many challenges. As a partner, Annie has been a friend, supporter, coach, guide, and far more than words can capture. The author is forever grateful for her limitless love and support and looks forward to all the new adventures ahead.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AA	Amino acid
AGP	Antibiotic growth promoters
AID	Apparent ileal digestibility
AIDE	Apparent ileal digestible energy
BA	Butyric acid
BMD	Bacitracin methylene disalicylate
bp	base pairs
BW	Body weight
BWG	Body weight gain
Ca	Calcium
CB	Calcium butyrate
CD	Crypt depth
cfu	colony-forming units
CLM	95% confidence limits for the mean
CP	Crude protein
CV	Coefficient of variation
d	day
DEGs	Differentially expressed genes
d_{gw}	Geometric mean diameter
DM	Dry matter
DNI	Digestible nutrient intake
FCR	Feed conversion ratio

FI	Feed intake
FITC-d	Fluorescein isothiocyanate dextran
FPD	Faith’s phylogenetic diversity index
FTU	Phytase unit
GE	Gross energy
KEGG	Kyoto Encyclopedia of Genes and Genomes
ME	Metabolizable energy
min	minutes
MMFA	Microbiota modulating feed additive
N	Newtons
NC	Negative control
NE	Necrotic enteritis
OF	Observed features
P	Phosphorus
PBS	Phosphate-buffered saline
PC	Positive control
PCoA	Principal coordinate analysis
PS	Particle size
r	Pearson correlation coefficients
s	seconds
TiO_2	Titanium dioxide
VH	Villus height
VH:CD	Villus to crypt depth ratio

I. INTRODUCTION

As global demand for sustainable protein sources rises, the poultry industry faces the challenge of maintaining affordable production while ensuring the health and performance of broilers. Optimizing broiler growth, nutrient utilization, and intestinal health requires continuous exploration of nutritional strategies that align with industry changes and consumer preferences. In response to the reduction or removal of antibiotic growth promoters (**AGP**), alternative feeding strategies are essential to sustain broiler performance and health while minimizing economic losses. Feeding strategies have focused on (1) feed additives and (2) diet manipulation.

Antibiotic growth promoters, such as bacitracin methylene disalicylate (**BMD**), remain in limited use in broiler diets, but offer benefits in managing pathogenic bacterial populations within the gut (Proctor and Phillips, 2019). Phytase and butyrate are possible alternatives to AGP and have been studied for their ability to enhance nutrient availability (Manobhavan et al., 2016; Walters et al., 2019) and to modulate intestinal physiology (Mátis et al., 2022; Deng et al., 2023), respectively. Phytase is routinely included in broiler diets to degrade phytate, enhance phosphorus digestibility, and reduce anti-nutritional effects of phytate (Selle et al., 2023). Butyrate-based feed additives, including calcium butyrate (**CB**), have potential to support intestinal integrity, enhance pathogen control, and improve nutrient absorption (Kaczmarek et al., 2016; Song et al., 2017; Giacomini et al., 2022). Interactions between phytase, CB, and BMD in broiler nutrition remain underexplored, particularly their combined effects on performance, nutrient digestibility, and intestinal health.

Altering dietary components such as calcium concentration and limestone particle size is another strategy to potentially improve broiler growth while mitigating disease challenges. Calcium is an important macro mineral with various biological roles, including skeletal development and metabolic functions (Proszkowiec-Weglarz and Angel, 2013). Limestone is the primary calcium source in most broiler diets, and its particle size can influence solubility (de Witt et al., 2006; Kim et al., 2019). However, excessive dietary calcium or highly soluble calcium sources may negatively affect nutrient utilization and increase severity of enteric diseases, such as necrotic enteritis (Paiva et al., 2013; Paiva et al., 2014). Interactions between dietary calcium levels and limestone particle sizes can influence calcium solubility and availability, impacting pathogen proliferation (Moran, 2014) and nutrient digestibility (Selle et al., 2009; Kim et al., 2018). Understanding how calcium levels and limestone particle size impact broiler performance and nutrient utilization, especially under enteric challenge conditions, is crucial for optimizing feeding strategies.

This dissertation consists of two studies that evaluated the impact of feed additives and dietary manipulation on broiler performance, nutrient utilization, and intestinal health under various disease conditions. The first study investigated the effects of varying phytase concentrations in combination with CB and BMD on performance, processing yields, and nutrient digestibility (Part 1), as well as intestinal permeability, jejunal histomorphometry, tight junction gene expression, and cecal microbiome diversity and function (Part 2) in broilers reared to 42 d of age. Both parts aimed to determine how phytase interacts with microbiota-modulating feed additives and whether these interactions influence broiler growth and intestinal physiology. The second study assessed the interactive effects of limestone particle size and calcium concentration on performance, tibia mineralization, and nutrient utilization during an enteric challenge with *Eimeria* spp. and *Clostridium perfringens* from 1 to 35 d of age. The objective of this study was to determine if varying dietary calcium levels and limestone particle sizes influence broiler growth and their resilience to an enteric disease.

II. LITERATURE REVIEW

ENTERIC CHALLENGES AND COMMON DIETARY INTERVENTIONS TO REDUCE DISEASE SEVERITY

Common enteric pathogens in broiler production and the shift away from antibiotic growth promoters

Broiler production is a major sector of U.S. animal agriculture, with around 9.15 billion broilers produced in 2023, valued at $42 billion (USDA-NASS, 2023). Alabama, a leading broiler-producing state, accounted for 1.2 billion broilers valued at $4.7 billion (USDA-NASS, 2023). As the global population is projected to reach 9 billion by 2050, food demand could increase by 56% (Maharjan et al., 2021; van Dijk et al., 2021), emphasizing the need for broiler meat as a sustainable and affordable protein source.

Advancements in genetics, nutrition, management, and the use of antibiotic growth promoters (**AGP**) have improved broiler production efficiency (Zuidhof et al., 2014; Maria Cardinal et al., 2019; Maharjan et al., 2021). However, enteric pathogens such as *Campylobacter*, *Clostridium perfringens*, *Eimeria* spp., *Escherichia coli*, *Enterococcus* spp., and *Salmonella* remain significant challenges for the poultry industry (Oakley et al., 2014; Fancher et al., 2020; Pietruska et al., 2023; Ribeiro et al., 2023). Among these, *Campylobacter* and *Salmonella* pose significant risks to human health (Oakley et al., 2014). Controlling these pathogens is difficult, as some of them (e.g., *C. perfringens*, *E. coli*, *Salmonella*) naturally inhabit the broiler gastrointestinal tract as commensals (Oakley et al., 2014; Clavijo and Flórez, 2018; Ribeiro et al., 2023). Additionally, their pathogenicity is often opportunistic, depending on predisposing factors that compromise broiler health (Oakley et al., 2014; Moore, 2016; Ribeiro et al., 2023).

Antibiotic growth promoters have been used to control enteric pathogens and enhance poultry production efficiency, weight gain, and feed conversion (Miles et al., 2006; Maria Cardinal et al., 2019). Various AGP have been incorporated in broiler diets, such as arsanilic acid, bacitracin methylene disalicylate (**BMD**), bacitracin zinc, bambermycins, chlotetracycline, lincomycin, oxytetracycline, penicillin, roxarsone, tylosin, and virginiamycin (Jones and Ricke, 2003). The observed improvements in weight gain and feed efficiency with AGP use may result from multiple underlying mechanisms. Early proposed mechanisms by which AGP enhance performance include: (i) improved nutrient absorption due to a thinner small-intestinal epithelium; (ii) reduced microbial competition, preserving nutrients for the host; (iii) decreased or eliminated subclinical infections caused by certain microorganisms; and (iv) lower production of growth-inhibiting toxins or metabolites by intestinal microflora (Feighner and Dashkevicz, 1987). Recent hypotheses by Miyakawa et al. (2024) suggest that AGP can support growth by influencing mitochondrial function, which helps maintain intestinal stability and immune regulation. This effect may reduce the energy demands associated with gut inflammation, leading to improved feed efficiency, disease resistance, and overall animal performance.

Several AGP used in broiler diets, including bacitracin, chlotetracycline, lincomycin, oxytetracycline, and penicillin, also have applications in human medicine (Jones and Ricke, 2003). Their use in poultry production has led to concerns about the emergence of antibiotic-resistant bacteria in both animals and humans, especially among gram-negative species like *Salmonella* spp. and *E. coli* (Butaye et al., 2003; Shah et al., 2017). Therefore, increasing concerns over antimicrobial resistance and shifting consumer demand have led most broiler producers to limit or discontinue AGP use (Huyghebaert et al., 2011). In the U.S., implementation of the Veterinary Feed Directive was also a primary factor contributing to the reduction in AGP usage (FDA, 2015). The reduced or complete removal of AGP in the European Union and U.S. poultry industries has shifted priorities toward alternative feeding strategies that support gastrointestinal health (Roth et al., 2019). However, despite these efforts, the decline in AGP use has been linked to potential reductions in broiler performance and an increased incidence of diseases such as coccidiosis and necrotic enteritis (**NE**) (Engster et al., 2002; Cervantes, 2015).

Pathophysiology of Eimeria spp., Clostridium perfringens, and necrotic enteritis

Eimeria spp., protozoan parasites of the phylum Apicomplexa, replicate in the intestinal tract, causing tissue damage that disrupts digestion, nutrient absorption, and feed intake while increasing susceptibility to secondary infections (McDougald and Fitz-Coy, 2008). Infection begins when the host sheds thick-walled oocysts in feces, which undergo sporulation to reach the infective stage. Upon ingestion, the oocyst wall is broken down in the gizzard, and sporozoites are released from sporocysts in the small intestine through the action of chymotrypsin and bile salts. These sporozoites invade epithelial cells, initiating replication that ultimately damages intestinal tissue (McDougald and Fitz-Coy, 2008; Tewari and Maharana, 2011). Several *Eimeria* species infect chickens, including *E. acervulina*, *E. brunetti*, *E. maxima*, *E. mitis*, *E. necatrix*, *E. praecox*, and *E. tenella* (López-Osorio et al., 2020). Pathogenicity varies among species, with *E. mitis* and *E. praecox* being the least pathogenic, while *E. brunetti, E. necatrix*, and *E. tenella* being the most pathogenic (López-Osorio et al., 2020). *Eimeria* spp. also have site-specific development within the chicken's gastrointestinal tract. *Eimeria acervulina* develops in the duodenum and jejunum, *E. maxima* in the duodenum, jejunum, and ileum, and *E. tenella* in the ceca (Tewari and Maharana, 2011; López-Osorio et al., 2020). The global economic burden of coccidiosis, caused by *Eimeria* spp., is estimated at $13 billion annually (Blake et al., 2020). Previous research has reported that broilers challenged with *Eimeria* spp. exhibited reduced growth (Watson et al., 2005; Amerah and Ravindran, 2015; Wang et al., 2018; Shi et al., 2024a) and lower tibia ash content (Watson et al., 2005; Akbari Moghaddam Kakhki et al., 2019; Shi et al., 2024a). Coccidia-infected broilers exhibited lower plasma calcium and phosphorus (**P**) levels, decreased tibia ash, and reduced bone strength (Oikeh et al., 2019). Additionally, an *Eimeria* challenge negatively affected nutrient utilization, as evidenced by decreased digestibility of nitrogen, starch, fat, and ileal digestible energy (Amerah and Ravindran, 2015).

Clostridium perfringens is a gram-positive, anaerobe that can range from 0 to 10^5 colony-forming units (**cfu**)/g in healthy birds, whereas in NE-affected birds, intestinal concentrations can reach 10^6 to 10^8 cfu/g, contributing to severe intestinal damage (Timbermont et al., 2011; Mora et al., 2020). Hussein et al. (2020) reported reduced growth when broilers were challenged with 4×10^8 cfu/g *C. perfringens*. However, NE is not solely linked to *C. perfringens* cell counts, as only strains with host-specific virulence factors can induce disease in poultry (Timbermont et al., 2011). *Clostridium perfringens* produces various enterotoxins, including α-toxin, β-toxin, ε-toxin, ι-toxin, CPE, and NetB and it is classified into toxinotypes A, B, C, D, E, F, and G based on toxin production (Rood et al., 2018). Types A, C, and G are the primary *C. perfringens* toxinotypes associated with NE in chickens (Opengart, 2008; Keyburn et al., 2010; Rood et al., 2018). Type A produces only α-toxin, type C produces α-toxin, β-toxin, and CPE, while type G produces α-toxin and NetB (Rood et al., 2018). These toxins can disrupt intestinal physiology, with α-toxin having phospholipase C and sphingomyelinase activity, leading to membrane damage, hemolysis, and necrosis (Sakurai et al., 2004). NetB forms pores in the phospholipid bilayer, compromising membrane integrity, facilitating ion influx, and causing osmotic cell lysis (Keyburn et al., 2010). However, α-toxin is considered less critical to NE pathogenesis compared to NetB (Keyburn et al., 2006; Keyburn et al., 2010).

Necrotic enteritis is a multifactorial enteric disease caused by *C. perfringens*, the main etiological agent (Fathima et al., 2022a). However, infection alone does not lead to disease since specific predisposing factors must facilitate *C. perfringens* overgrowth (Moore, 2016). Several predisposing factors can promote the growth of *C. perfringens,* including *Eimeria* infection, removal of coccidiostats or AGP, environmental and managemental conditions (e.g., wet litter, temperature stress), and dietary components (e.g., high protein levels, mycotoxins) (Dahiya et al., 2006; Moore et al., 2016; Fathima et al., 2022a). These factors promote conditions favorable for NE by altering gut structure, modulating immune function, disrupting gastrointestinal microbiota, and increasing *C. perfringens* proliferation (Moore, 2016). The clinical form of NE presents with depression, ruffled feathers, diarrhea, huddling, anorexia, and a sudden rise in flock mortality (To et al., 2017). In contrast, subclinical NE results in chronic intestinal damage that reduces feed intake (**FI**) and weight gain, leading to decreased performance and an increased feed conversion ratio (**FCR**) without significant mortality (Palliyeguru and Rose, 2014; To et al., 2017; Emami and Dalloul, 2021). Although subclinical NE does not cause a sudden increase in mortality, its diagnosis is challenging, making it the most economically significant form in the poultry industry (Dahiya et al., 2006; Palliyeguru and Rose, 2014). Research using NE challenge models continues to demonstrate its negative effects on broiler performance (Zanu et al., 2020a), energy digestibility (M'Sadeq et al., 2015), and tibia ash (Zanu et al., 2021).

Enteric pathogens disrupt multiple physiological processes, leading to reduced broiler performance. Growth reduction in response to an enteric challenge may be linked to decreased FI, potentially driven by immune activation (Dantzer, 2004). These pathogens impair nutrient digestibility (Williams, 2005; Guo et al., 2014; Rochell et al., 2016) by damaging the intestinal mucosa (Wu et al., 2016; Kinstler et al., 2024). Additionally, enteric diseases can alter bile acid concentrations, reducing fat digestibility (Bansal et al., 2021). Furthermore, enteric diseases can impair bone resorption (Tompkins et al., 2023) and nutrient absorption (Amerah and Ravindran, 2015; Rochell et al., 2016), contributing to compromised skeletal mineralization.

Nutritional strategies to mitigate enteric challenges

The use of AGP has historically been an effective strategy in broiler nutrition by improving growth rate, reducing FCR, and promoting bird health through intestinal microbiota modulation and disease control (Engberg et al., 2000; Miles et al., 2006; Mehdi et al., 2018; Maria Cardinal et al., 2019). Studies have reported improved broiler performance with AGP supplementation compared to AGP-free diets (Murugesan et al., 2015; Manafi et al., 2017; Li et al., 2022) and even certain AGP alternatives, such as butyrate-based feed additives (Naghizadeh et al., 2022a; Naghizadeh et al., 2022b). While AGP use has been removed or limited in many regions (Mehdi et al., 2018), BMD is still used in poultry production and will be the focus of this dissertation.

Bacitracin methylene disalicylate, a polypeptide antibiotic, has been widely used as an AGP in poultry diets, primarily targeting gram-positive bacteria such as *Streptococci*, *Staphylococci*, *Clostridia*, *Fusobacterium*, and *Actinomyces* (Proctor and Phillips, 2019). By controlling *C. perfringens* (Stutz et al., 1983), reducing pathogenic bacterial load, and modulating intestinal microbiota (Casewell et al., 2003; Crisol-Martínez et al., 2017; Li et al., 2022), BMD can support gut health and nutrient utilization. Its use was shown to enhance crude protein (**CP**) digestibility compared to AGP-free diets (Murugesan et al., 2015; Manafi et al., 2019), likely due to its role in shaping gut microbiota and improving intestinal morphology (Murugesan et al., 2014; Murugesan et al., 2015; Kumar et al., 2019). By inhibiting bacterial cell wall synthesis (Smith and Weinberg, 1962), BMD may reduce the availability of undigested protein and amino acids (**AA**), limiting substrates that could otherwise support pathogenic proliferation and toxin production in the lower digestive tract (Apajalahti and Vienola, 2016). However, some studies report that BMD had no influence on microbial diversity (Neumann and Suen, 2015; Proctor and Phillips, 2019) or intestinal morphology (Adhikari et al., 2023), indicating inconsistencies in its effects.

With the limitations in AGP usage, various nutritional strategies have been explored as potential alternatives. To be considered an AGP alternative, a nutritional strategy must exhibit antimicrobial properties while supporting growth, immunity, and overall health, mimicking the benefits of AGP. However, the mechanisms and modes of action of many AGP alternatives remain unclear (Wickramasuriya et al., 2024). In poultry, AGP alternatives include probiotics, prebiotics, phytogenics, organic acids, bacteriophages, symbiotics, enzymes, antimicrobial peptides, vaccines, and heavy metals and clays (Gadde et al., 2017; Ayalew et al., 2022; Wickramasuriya et al., 2024). Several nutritional strategies, including probiotic (Ritzi et al., 2014), phytogenic (Wati et al., 2015), and organic acid supplementation (Pereira et al., 2015), have been shown to mitigate the adverse effects of enteric challenges on broiler performance. Furthermore, nutritional strategies incorporating AGP alternatives such as probiotics (Cao et al., 2013), bacteriophages (Sarrami et al., 2023), and symbiotics (Mora et al., 2019) have been reported to positively influence intestinal microbiota, potentially reducing the severity of enteric diseases. The following sections will explore two nutritional strategies as alternatives to AGP: (1) the use of phytase and butyrate-based feed additives to enhance performance and modulate intestinal microbiota and (2) dietary manipulation, specifically examining the effects of calcium levels and limestone particle size on broiler growth and enteric disease severity.

PHYTASE AND BUTYRATE-BASED FEED ADDITIVES IN BROILER NUTRITION

Phytase

Phosphorus is critical for bone formation and metabolism, as well as energy production, nutrient metabolism, muscle function, skeletal development, blood homeostasis, and fatty acid transport (Leeson and Summers, 2001a; Humer et al., 2015). However, over 60% of P in cereal and legume seeds is bound to phytic acid, limiting its bioavailability to approximately 20 to 30% (Reddy et al., 1982; Ravindran et al., 1994; Humer et al., 2015; Fernandes et al., 2019; Taliman et al., 2019). Nelson et al. (1968 and 1971) established the importance of exogenous phytase in poultry production, and subsequent research led to the commercial launch of Natuphos® (BASF AG, Ludwigshafen, Germany) in 1991 (Chen et al., 2014). Exogenous phytase is routinely added to broiler diets to hydrolyze phytate, releasing bound nutrients and mitigating its anti-nutritional effects (Selle et al., 2000; Selle and Ravindran, 2007; Dersjant-Li et al., 2015; Selle et al., 2023). Phytic acid ($C_6H_{18}O_{24}P_6$), its mixed-salt derivative phytate, and its mineral complex phytin (containing potassium, magnesium, and calcium) represent the primary substrate of phytase in plant-based feedstuffs (Selle and Ravindran, 2007; Morales et al., 2016). Although phytate is considered an anti-nutrient, it contributes to meeting broiler P requirements, reducing reliance on inorganic P sources (Ibrahim et al., 1999), which could be depleted within 50 years (Lei et al., 2007).

Phytate readily chelates minerals, forming complexes with up to 5 calcium atoms, which can bind one-third of dietary calcium in the digesta (Selle et al., 2009). Calcium-phytate complex formation occurs primarily in the small intestine at pH ≥ 5 and is exacerbated by high dietary calcium levels and wider calcium:available P ratios, which can increase pH in the crop, proventriculus, and gizzard (Selle et al., 2009). Additionally, phytate's binding affinity shifts with pH, forming mineral complexes at neutral pH while binding AA, proteins, and starch under acidic conditions (Fernandes et al., 2019). Its phosphate groups exhibit specificity for cationic AA (Wang and Guo, 2021), forming ternary protein-phytate complexes with positively charged proteins (Selle et al., 2012). Beyond its role in nutrient binding, phytate stimulates gastric HCl and pepsin secretion, which is countered by increased sodium bicarbonate and mucus production in the small intestine (Selle et al., 2009). This can reduce sodium availability for monosaccharide and AA absorption via the Na^+-dependent transport system (Glynn, 1993; Leeson and Summers, 2001b; Selle et al., 2009), potentially increasing undigested fermentable sugars, favoring pathogenic bacterial proliferation in the ceca and large intestine (Leeson and Summers, 2001b; Singh and Kim, 2021). Phytate can also inhibit endogenous digestive enzymes such as trypsin (Singh and Krikorian, 1982).

Phytase (myo-inositol hexakisphosphate phosphohydrolase) is an enzyme widely used in poultry diets to hydrolyze phytate, releasing inorganic phosphate and lower inositol phosphate esters while mitigating phytate’s anti-nutritional effects (Pallauf and Rimbach, 1997; Selle et al., 2023). Phytase activity is defined based on specific assay conditions, and as a result, a universally standardized unit has not been established (Selle and Ravindran, 2007). However, Engelen et al. (1994) defined 1 phytase unit (**FTU**) as the release of 1 µmol of inorganic ortho-phosphate per minute from 0.0051 mol/L sodium phytate at 37°C and pH 5.5. The efficacy of exogenous phytase is influenced by the bird’s gastrointestinal environment, including pH variations across different segments of the digestive tract, which can affect phytase’s activity and its ability to hydrolyze phytate effectively. Typical pH values range from 7.0 to 7.5 in the mouth, 4.5 in the crop, 2.5 in the proventriculus and gizzard, 6.0 to 6.8 in the duodenum, 5.8 to 6.8 in the jejunum, and 5.7 to 5.9 in the ceca, with fluctuations influenced by dietary composition and microbial activity (Leeson and Summers, 2001b; Mateos et al., 2012; Singh and Kim, 2021). Since phytate hydrolysis primarily occurs in the crop, proventriculus, and gizzard, maximizing enzymatic activity in these compartments is critical for reducing the formation of insoluble complexes and enhancing P retention (Adeola and Cowieson, 2011).

Phytase supplementation can improve broiler performance and nutrient utilization by increasing P availability and reducing the inhibitory effects of phytate (Nelson et al., 1968; Nelson et al., 1971; Perney et al., 1993; Liu et al., 2009; Ptak et al., 2015; Ajuwon et al., 2020). In diets with low available P, broilers fed a diet with phytase exhibited higher body weights (**BW**), improved FCR, and enhanced nutrient digestibility compared to those fed a phytase-deficient diet (Santos et al., 2008; Liu et al., 2010; Walters et al., 2019). The degree of improvement in nutrient digestibility can depend on phytase levels. For instance, supplementing broilers with 500 FTU/kg of phytase increased calcium and P digestibility, while increasing the dose to 2,500 FTU/kg further increased P digestibility (Manobhavan et al., 2016). In addition to increasing mineral digestibility, phytate degradation reduces its anti-nutritional effects, including endogenous nitrogen losses (Cowieson et al., 2004; Cowieson et al., 2009). Beyond P release, phytase mitigates phytate's capacity to complex with other minerals, AA, and vitamins, improving nutrient availability and overall performance (Adeola and Cowieson, 2011). Its extra-phosphoric effects include inositol release, enhanced apparent metabolizable energy (**ME**), and improved AA digestibility (Liu et al., 2008a; Gehring et al., 2013; Fernandes et al., 2019; Kriseldi et al., 2021a). The energy-sparing effect of phytase varies from 0 to 120 kcal/kg ME, depending on dietary energy levels and the inclusion rate of phytase (Adeola and Cowieson, 2011). Proposed mechanisms for the energy sparing effect of phytase include increased nutrient solubility, reduced endogenous energy loss, enhanced sodium-dependent nutrient transport, and degradation of fibrous material (Adeola and Cowieson, 2011). Additionally, phytase supplementation enhances fat (Liu et al., 2010) and energy digestibility (Leyva-Jimenez et al., 2019) in broilers, primarily by reducing phytate's anti-nutritional effects, minimizing endogenous losses, and releasing bound nutrients (Ravindran et al., 2000; Camden et al., 2001; Santos et al., 2008; Selle et al., 2009; Walters et al., 2019). Phytase can also support muscle deposition by increasing CP (Santos et al., 2008; Liu et al., 2009) and AA digestibility (Gehring et al., 2013; Walters et al., 2019).

Phytase is primarily used to degrade phytate and increase P availability; however, it can also indirectly influence intestinal health. Phytase supplementation can promote *Lactobacillus* colonization in the jejunum and ileum (Ptak et al., 2015; Moita et al., 2021), modulate short-chain fatty acid concentrations, and alter villus height (**VH**) (Ptak et al., 2015; Moita et al., 2021). Additionally, phytase may impact systemic immunity by increasing percentages of $CD4^+$ $CD8^+$ T lymphocyte subsets, improving levels of intestinal secretory IgA, and enhancing antibodies against Newcastle disease virus vaccine (Liu et al., 2008b). However, Liu et al. (2008b) observed that immune function was not further improved by increasing phytase supplementation from 500 to 1,000 FTU/kg. Phytase reduces undigested protein and nitrogen flow, limiting the substrate available for pathogenic bacteria (Qaisrani et al., 2015). Additionally, by lowering endogenous nitrogen losses, phytase may further restrict proliferation of these bacteria (Ptak et al., 2015; Moita et al., 2021). Complete phytate dephosphorylation releases inositol (Kriseldi et al., 2021b), which can further influence gut microbiota (Yan et al., 2024). Phytase at 1,500 FTU/kg was shown to decrease Gly, Ser, and Thr metabolism in the ileal microbiota (Siegert et al., 2021). Although some studies suggest phytase may enhance intestinal histomorphometry (Layter et al., 2021; Moita et al., 2021) and alter intestinal gene expression (Zanu et al., 2020b), other researchers reported that phytase does not affect intestinal permeability (Zanu et al., 2020b; Zanu et al., 2020c).

Butyrate-based feed additives

Butyric acid (**BA**) is a 4-carbon carboxylic acid with a terminal carboxyl group (-COOH). Butyric acid is characterized by a molecular weight of 88.12 g/mol, a density of 0.958 g/mL, and a pKa of 4.82. In solution, the carboxyl hydrogen dissociates, forming a butyrate ion ($CH_3CH_2CH_2COO^-$) (Ahsan et al., 2016). Butyrate is a short-chain fatty acid naturally produced in the gastrointestinal tract through microbial fermentation of carbohydrates (Guilloteau et al., 2010; Levy et al., 2015). Butyric acid or butyrate-based feed additives have been evaluated as promising alternatives to AGP (Deepa et al., 2018; Ayalew et al., 2022; Giacomini et al., 2022). Previous broiler research has focused on BA (Leeson et al., 2005), sodium butyrate (Bortoluzzi et al., 2017), calcium butyrate (Adhikari et al., 2023), monobutyrin (Bedford et al., 2017), tributyrin (Bedford et al., 2018), and butyrate glyceride mixtures (Yin et al., 2016). However, this review will primarily focus on butyrate-based organic acid salts. Butyrate-based organic acid salts, including sodium and calcium butyrate, contain sodium or calcium replacing the carboxyl hydrogen, respectively (Ahsan et al., 2016).

Following ingestion, butyrate-based organic acid salts release BA, which will remain largely undissociated due to its pKa (4.82) being higher than the pH of the proventriculus and gizzard (pH = 2.5 to 3.5) (Ravindran, 2013; Ahsan et al., 2016). Dissociation increases as BA moves into the duodenum (pH = 5 to 6), jejunum (pH = 6.5 to 7), and ileum (pH = 7 to 7.5) (Ravindran, 2013), yielding butyrate and hydrogen ions.

Butyric acid and butyrate ions can diffuse into enterocytes, supporting villus development and epithelial turnover (Hu and Guo, 2007; Ahsan et al., 2016). Butyrate-based feed additives can also control bacteria through direct and indirect bactericidal effects. In the small intestines, direct bactericidal effects require BA to be undissociated while the indirect effects require BA dissociated (Ahsan et al., 2016). For direct bactericidal effects, undissociated BA diffuses across bacterial cell walls (Van Immerseel et al., 2006), disrupting intracellular homeostasis and exerting bactericidal effects (Warnecke and Gill, 2005). This can lead to enzyme denaturation and cell death (Roe et al., 2002). In terms of indirect bactericidal effect, BA dissociates reducing intestinal pH, potentially promoting the proliferation of beneficial lactic acid-producing bacteria, which have higher acid tolerance (Lankaputhra and Shah, 1995; Jin et al., 1998). These commensal bacteria can compete with pathogens for nutrients and intestinal receptors (Rolfe, 2000; Servin, 2004; Fathima et al., 2022b) and secrete antimicrobial compounds (Gibson and Wang, 1994; Joerger, 2003).

Research on butyrate-based feed additives suggests mixed effects on broiler performance. Some studies report improved performance (Levy et al., 2015; Kaczmarek et al., 2016; Raza et al., 2019) or no difference compared to AGP (Leeson et al., 2005; Kaczmarek et al., 2016; Sikandar et al., 2017), while others found no impact on performance (Adhikari et al., 2023), processing weights (Raza et al., 2019), or processing yields (Leeson et al., 2005). However, butyrate-based feed additives have been shown to increase carcass and breast yield (Dehghani-Tafti and Jahanian, 2016; Adhikari et al., 2023) and reduce abdominal fat (Panda et al., 2009). Inconsistent responses could be attributed to dosing level, as shown by Sizmaz et al. (2022), where inclusion of a coated

BA product at 400 and 800 ppm increased d 42 BW of broilers, but only 800 ppm improved FCR. Furthermore, BA is naturally volatile, corrosive, and rapidly absorbed in the upper gastrointestinal tract (Ahsan et al., 2016; Sizmaz et al., 2022). Uncoated BA products may have no effect on performance at higher doses (2,000 and 4,000 ppm) and could even reduce feed consumption at 4,000 ppm (Leeson et al., 2005). To ensure effective delivery to the lower gastrointestinal tract, butyrate salts or coated products are commonly used in diets (Ahsan et al., 2016; El-Saadony et al., 2022; Zhao et al., 2022). Butyrate may also enhance CP, fat, and energy digestibility (Kaczmarek et al., 2016; Liu et al., 2017; Nari and Ghasemi, 2020). These beneficial effects have been linked to modulation of intestinal microbiota (Panda et al., 2009; Wu et al., 2018), enhanced mineral solubility and absorption (Adil et al., 2010), and improved intestinal morphology (Sikandar et al., 2017; Raza et al., 2019).

Reviews by Guilloteau et al. (2010), Deepa et al. (2018), and El-Saadony et al. (2022) highlight that BA supplementation can improve pathogen control, promote beneficial bacteria, lower luminal pH, enhance nutrient digestibility, and reduce oxidative stress. Butyrate-based feed additives can influence microbial composition (e.g., alpha diversity) and activity (Ficagna et al., 2022; Wan et al., 2022; Deng et al., 2023), including reducing *Salmonella* colonization and shedding (Van Immerseel et al., 2005; Fernández-Rubio et al., 2009; Onrust et al., 2020). Furthermore, butyrate improved *Salmonella enteritidis* resistance by stimulating host defense peptide gene expression (Sunkara et al., 2011). While butyrate can alter the gut microbiota (Wan et al., 2022), Naghizadeh et al. (2022a) reported no significant differences in bacterial counts (*Enterococci*, *C. perfringens*, and *Lactobacillus salivarius*) between broilers fed butyrate-supplemented and control diets. Butyrate-based feed additives have also been reported to have no effect on cecal microbiota alpha diversity (Wu et al., 2018; Melaku et al., 2024) or beta diversity (Liu et al., 2022; Melaku et al., 2024).

Further research indicates benefits of BA on intestinal health (Kaczmarek et al., 2016; Song et al., 2017; Mátis et al., 2022). Butyrate-based feed additives can enhance intestinal morphology by increasing VH and VH to crypt depth ratio (**VH:CD**) (Chamba et al., 2014; Ficagna et al., 2022; Deng et al., 2023), exhibit anti-inflammatory properties by modulating pro- and anti-inflammatory cytokines (Zhang et al., 2011; Zou et al., 2019), and improve intestinal integrity (Song et al., 2017; Mátis et al., 2022). Although butyrate has improved intestinal integrity, it was shown to have no effect on intestinal permeability (Naghizadeh et al., 2022b). Additionally, butyrate can exhibit immunomodulatory effects (Zhou et al., 2014). Bortoluzzi et al. (2017) reported that sodium butyrate altered cecal microbiota structure, composition, and predicted function, which possibly contributed to the upregulation of immune-related genes, including ubiquitin-editing enzyme A20 and IL-6. Microencapsulated sodium butyrate has been reported to restore performance and intestinal integrity in broilers infected with NE by regulating the expression of tight junction proteins (claudin-1, claudin-4, occludin, and zonula occludens-1), mucin-2, and LEAP-2 (Song et al., 2017). The efficacy of butyrate mitigating the negative effects of enteric disease may depend on its place of release in the gastrointestinal tract. Liu et al. (2019) observed that sodium butyrate targeting the anterior intestinal tract improved broiler performance.

Interactions between phytase and butyrate-based feed additives

Organic acids can lower digestive tract pH (Esmaeilipour et al., 2011; Han et al., 2023), potentially limiting calcium-phytate formation, optimizing phytase activity (Selle et al., 2009; Vieira et al., 2018), and exerting antimicrobial effects (Biggs and Parsons, 2008). Combining organic acids and phytase may also serve as an alternative to AGP, enhancing broiler growth and nutrient utilization (Vieira et al., 2017; Vieira et al., 2018). Butyrate-based feed additives combined with phytase may enhance intestinal morphology by increasing VH and reducing CD (Layter et al., 2021), potentially improving performance and feed efficiency (Kaczmarek et al., 2016). However, responses appear inconsistent, as Layter et al. (2021) observed that increasing phytase from 750 to 1,500 FTU/kg with sodium butyrate increased jejunum CD and ileum VH, suggesting intestinal segment-specific responses. Therefore, given the limited research, the extent to which phytase and butyrate-based feed additive interactions influence intestinal histomorphometry remains unclear.

Knowledge gaps remain regarding the optimal phytase concentration when combined with organic acids. Vieira et al. (2017) reviewed the benefits of combining citric acid and phytase, reporting increased weight gain and improved bone mineralization. Research has primarily focused on phytase and citric acid interactions, likely due to citric acid's greater ability to lower gastrointestinal pH compared to BA, which has a higher pKa and may be less effective in this regard (Vieira et al., 2018). Currently, there is limited research evaluating the interaction between phytase and butyrate-based feed additives. Given the widespread usage of phytase in broiler diets and increasing interest in butyrate-based feed additives as AGP alternatives, further investigation is warranted to understand their interactions.

CALCIUM CONCENTRATIONS AND LIMESTONE PARTICLE SIZE IN BROILER NUTRITION

Calcium

Calcium is a critical macro mineral in broiler nutrition, serving as a structural component of bones, an intracellular messenger, and a cofactor for various enzymes, with its essential roles in broiler growth and physiology well-documented (Adedokun and Adeola, 2013; Proszkowiec-Weglarz and Angel, 2013; David et al., 2023). Approximately 99% of calcium is stored in bones as hydroxyapatite, with the remaining 1% found in intracellular and extracellular compartments (Proszkowiec-Weglarz and Angel, 2013; David et al., 2023). A small fraction of extracellular calcium (~ 0.1% of total) circulates as free ions or is bound to proteins and anions, though only ionized calcium (Ca^{2+}) is physiologically active (Parsons and Combs, 1981; David et al., 2023). Like mammals, growing chickens maintain a total plasma calcium level of approximately 10 mg/dL (Diaz et al., 1997). During reproduction, hens can exhibit total plasma calcium levels exceeding 20 mg/dL; however, homeostatic regulation preserves ionized calcium concentrations within a narrow range of 1.2 to 1.3 mmol (Diaz et al., 1997). Calcium homeostasis is maintained through feedback mechanisms involving parathyroid hormone, active vitamin D_3 (1,25-dihydroxyvitamin D_3), and calcitonin (Proszkowiec-Weglarz and Angel, 2013; David et al., 2023). Receptors in the small intestine, bone, and kidneys, such as vitamin D receptors (Pedrozo et al., 1999) and calcium-sensing receptors (Diaz et al., 1997; Brown, 2007), further contribute to this regulatory process. During hypocalcemia, parathyroid hormone promotes bone calcium mobilization, renal calcium resorption, and stimulates renal synthesis of 1,25$(OH)_2D_3$, which enhances both intestinal calcium absorption and further renal resorption (Leeson and Summers, 2001a). Increased intestinal calcium absorption involves elevated expression of calbindin, a calcium-binding protein that facilitates intracellular calcium transport across enterocytes (Nemere et al., 1991; Li et al., 2012; Proszkowiec-Weglarz and Angel, 2013). Conversely, calcitonin predominates under normal or hypercalcemic conditions, inhibiting osteoclast-mediated bone resorption and counteracting parathyroid hormone activity (Eliam et al., 1988; Naveh-Many and Silver, 1988; Leeson and Summers, 2001a).

Calcium must be solubilized for absorption in the gastrointestinal tract (David et al., 2023). Calcium solubility is pH-dependent, with lower pH promoting calcium solubilization (Guinotte et al., 1995; Walk et al., 2012a), emphasizing the role of the proventriculus and gizzard (pH 2.5 to 3.5) in this process (Ravindran, 2013; David et al., 2023). In contrast, the higher pH of the small intestine (> 5; Ravindran, 2013) can reduce calcium solubility (Walk et al., 2012a) and promote the precipitation of calcium, phytate, and phosphorus (Selle et al., 2009). Ionized calcium is absorbed via passive or active transport (Buckley and Bronner, 1980; Auchère et al., 1998; Felix, 1998), with absorption primarily occurring in the jejunum and ileum (Mutucumarana et al., 2014). Plasma calcium levels influence calcium absorption pathways. Under high calcium states, passive paracellular transport is favored, allowing calcium to diffuse between enterocytes (Buckley and Bronner, 1980). In contrast, active transcellular transport becomes more prominent when calcium levels are low (Auchère et al., 1998).

Additional factors affecting calcium solubilization and absorption include the calcium to available P ratio (Gautier et al., 2017), calcium-phytate complex formation (Selle et al., 2009), and exogenous phytase supplementation (Broch et al., 2018). For example, lower dietary calcium concentrations may minimize calcium-phytate interactions, enhancing phytase activity and improving nutrient digestibility (Sebastian et al., 1996; Selle et al., 2009). Shi et al. (2022) reported that reducing dietary calcium and available P from 0.90 and 0.45% to 0.75 and 0.30%, respectively, increased calcium and P digestibility and phytate degradation. Broilers may regulate calcium intake to meet physiological demands (Joshua and Mueller, 1979; Wilkinson et al., 2014), while birds fed lower calcium diets could adapt over time to improve mineral utilization (Yan et al., 2005). Excessive dietary calcium can impair nutrient digestibility (Shafey and McDonald, 1991; Mutucumarana et al., 2014; Tancharoenrat and Ravindran, 2014), reinforcing the importance of optimizing calcium levels for both skeletal integrity and performance. Insoluble calcium soap formation is a possible consequence of high dietary calcium, potentially limiting utilization of calcium and energy (Tancharoenrat and Ravindran, 2014). The impact of dietary calcium on energy utilization remains inconsistent, with varying responses reported (Walk et al., 2012b; Tancharoenrat and Ravindran, 2014; Akter et al., 2018). Conversely, calcium deficiency or an imbalanced calcium to P ratio can impair broiler growth and bone development (Li et al., 2020; Hu et al., 2021).

While adequate dietary calcium is essential for broiler development and physiological function, excessive calcium or highly soluble calcium sources may negatively impact growth and health, particularly under enteric challenge conditions (Paiva et al., 2013; Paiva et al., 2014). Shi et al. (2022) reported that unvaccinated broilers fed a diet with 0.15% less calcium and P had lower body weight gain (**BWG**) from d 0 to 21 compared to those fed a positive control (**PC**) diet with higher calcium and P. However, when broilers received a coccidial vaccine, BWG from d 0 to 21 decreased in the PC group, making it comparable to that of broilers fed the reduced calcium and P diet. High calcium levels may also contribute to NE pathogenicity by promoting *C. perfringens*' toxin production (Keyburn et al., 2010; Fathima et al., 2022a) and increasing calcium-phytate complex formation (Selle et al., 2009), leading to undigested nutrients that can serve as substrates for *C. perfringens* proliferation (Moran, 2014). Performance responses to dietary calcium under NE challenges can vary. Zanu et al. (2020a) reported reduced BWG and FI from d 0 to 42 in broilers fed high calcium diets under a subclinical NE challenge. Paiva et al. (2013) reported similar BWG, FI, and FCR between broilers fed a diet with 0.60 and 0.90% calcium under a clinical NE challenge. In a separate study, Paiva et al. (2014) reported higher mortality (d 0 to 19 and 0 to 35) in broilers challenged with clinical NE when fed a diet containing 0.90% calcium compared to 0.60%. In the absence of an enteric disease, Walk et al. (2012c) observed comparable performance between broilers fed 0.64 and 1.03% dietary calcium. Broiler performance responses to calcium concentration during an enteric challenge may vary depending on the duration of dietary treatments and the timing of post-infection measurements (Shi et al., 2024b).

Calcium supplementation can influence tibia mineralization, though the response may depend on enteric health status. Under subclinical NE conditions, Zanu et al. (2020d) reported higher tibia breaking strength (d 16) with increased dietary calcium, while Paiva et al. (2014) observed higher tibia ash content on d 35 in broilers fed diets with 0.90% calcium compared to broilers fed diets with 0.60% calcium during a clinical

NE challenge. In unchallenged broilers, Walk et al. (2012b) observed no effect of calcium level (0.45 vs. 0.90%) on tibia ash. Regarding nutrient digestibility, Zanu et al. (2020a) reported that broilers challenged with subclinical NE and fed a high calcium diet had lower apparent ileal digestibility (**AID**) of CP, carbon, potassium, and zinc compared to unchallenged broilers. The same study found that higher dietary calcium increased intestinal pH across multiple segments (gizzard, jejunum, ileum, and ceca), regardless of disease status, potentially affecting mineral solubilization. During a clinical NE challenge, increasing dietary calcium from 0.60 to 0.90% lowered d 21 AID of P and calcium when the diet contained limestone and 1,000 FTU/kg of phytase (Paiva et al., 2013). This effect was not observed without phytase (0 FTU/kg), highlighting the interaction between dietary calcium and phytase during an enteric challenge. Additionally, higher calcium levels and NE occurrence reduced calbindin expression (Zanu et al., 2020b), whereas NE prevented the serum calcium increase observed with higher dietary calcium in unchallenged broilers (Zanu et al., 2020d).

Due to the low cost and widespread availability of calcium sources, unintentional over-supplementation is common, highlighting the need for stricter control (Walk, 2016; Li et al., 2017; David et al., 2023). Concerns over excessive calcium levels in broiler diets (Walk, 2016) have prompted interest in adopting a digestible calcium system (Walk et al., 2021). However, Walk et al. (2021) hypothesized that limited adoption of a digestible or available calcium formulation system in the industry stems from several factors, including the scarcity of digestible calcium values for common feed ingredients, variability in analytical data, low cost of calcium sources, and complex interactions between calcium, digestible P, vitamin D_3, and phytate. Given that more than 3 decades have passed since the last nutrient requirements for poultry were established (National Research Council, 1994), understanding how modern broilers utilize dietary calcium is critical.

Limestone particle size

Limestone is the primary and most economical calcium source in broiler diets, providing over 50% of dietary calcium (Kim et al., 2019; David et al., 2023). Composed mainly of calcite (calcium carbonate; $CaCO_3$), limestone may also contain impurities such as calcium oxide, aragonite, and dolomite (David et al., 2023). Limestone can form through precipitation, secretion by marine organisms, accumulation of shell fragments, and calcite-mediated cementation of sand or mud (David et al., 2023). Dolomitic limestone is rarely used in poultry diets due to its lower calcium (20.5%) and higher magnesium content (12%) (Stillmak and Sunde, 1971) compared to calcitic limestone. A recent global review reported that fine limestone (< 1000 µm) contained between 33.3 and 39.7% calcium (average = 37.8%), while coarse limestone (> 1000 µm) ranged from 34.7 to 40.0% (average = 38.0%) (Gilani et al., 2022). Mussini et al. (2019) reported that limestone used by the U.S. poultry industry contained an average calcium level of 37.52%. Interestingly, the calcium content of limestone has remained relatively stable over the past 5 decades, as Reid and Weber (1976) observed an average of 37.8%.

Beyond its calcium content, limestone particle size can vary significantly by region (Gilani et al., 2022). Across all sampled regions, average particle size of fine limestone was 288 µm (range = 37.7 to 991.9 µm), while coarse limestone was 1,689 µm (range = 301.6 to 3,067.9 µm). Gilani et al. (2022) reported that Asia had the smallest average fine limestone particle size (153.9 µm), whereas North America (U.S. and

Canada) had the largest (356.3 µm). In North America, the coefficient of variation for the 151 limestone samples was 55.2%, highlighting high variability with particle size ranging from 37.7 to 992.0 µm. In a separate study, limestone samples from the U.S. varied in particle size, ranging from 59 to 495 µm (fine) and 848 to 2,803 (coarse) (Mussini et al., 2019). Collected from 26 quarries across 14 states, these samples highlight the variability in limestone particle size within the U.S.

Smaller limestone particles exhibit higher *in vitro* solubility (de Witt et al., 2006; Kim et al., 2019) but lower *in vivo* solubility (de Witt et al., 2006). In addition, limestone particle size can influence *in vitro* phytate-P hydrolysis (Manangi and Coon, 2007). *In vitro* testing showed that fine limestone had an average solubility of 68.4% after 5 min, with values ranging from 18.8 to 99.4%. Coarse limestone exhibited an average solubility of 65.5% after 30 min, with a range of 23.2 to 96% (Gilani et al., 2022). Gilani et al. (2022) determined that solubility correlated more strongly with fine limestone ($R^2 = 0.52$) than with coarse limestone ($R^2 = 0.09$). Similarly, Mussini et al. (2019) reported that fine limestone had a higher correlation ($R^2 = 0.641$) with solubility compared to coarse limestone ($R^2 = 0.3003$). The authors reported that fine limestone had a solubility range of 32.83 to 92.25% at 5 min, while coarse limestone ranged from 17.47 to 70.18% at 30 min. Fine and coarse limestone reached a similar solubility range (87 to 99%) after 30 and 150 min, respectively (Mussini et al., 2019). Increasing the *in vivo* solubility of the calcium source by using a coarser particle size can also affect digestive tract pH, with coarse limestone lowering gizzard pH compared to fine limestone (Kim et al., 2018; Lee et al., 2021). However, this effect varies depending on calcium concentration and the inclusion of exogenous phytase. Understanding how limestone particle size affects its solubility is crucial, as increased calcium solubility may promote calcium-phytate complex formation (Selle et al., 2009), potentially interfering with nutrient utilization (Bedford and Rousseau, 2017).

Kim et al. (2018) reported that pulverized limestone (< 75 µm) had higher *in vitro* solubility (96.9 vs. 74.3% at 5 min; 99.1 vs. 89.4 at 10 min) and was more responsive to calcium concentration and phytase activity than particulate limestone (402 µm). Additionally, pulverized limestone negatively affected AID of calcium and P compared to particulate limestone. Similarly, Li et al. (2021) showed that broilers fed a diet containing 800 µm limestone had higher standardized ileal digestibility and digestible calcium and P than those fed a diet with 151 µm limestone. In contrast, Majeed et al. (2020) observed that coarse limestone (900 µm) reduced AID of calcium and P compared to fine limestone (190 µm). Lee et al. (2021) further observed lower soluble calcium and P in the gizzard with coarse limestone compared to fine. These conflicting results may be influenced by calcium concentration and exogenous phytase, which can interact with limestone particle size (Majeed et al., 2020; Li et al., 2021). Although limestone particle size affects its solubility, it is not a reliable predictor of *in vivo* AID of calcium or P. Factors such as geological origin, chemical and physical properties, and particle size distribution influence calcium solubility (Kim et al., 2019). In addition to calcium digestibility, the particle size of calcium sources has also been shown to influence AID of copper, manganese, and strontium (Bradbury et al., 2018). Majeed et al. (2020) reported that fine limestone (190 µm) improved AA digestibility compared to coarse limestone (900 µm).

Although limestone particle size can affect nutrient digestibility, its impact on broiler performance is limited. Manangi and Coon (2007) observed that broilers achieved the highest BWG when fed a diet with either 137 or 299 µm limestone, while the lowest BWG and highest mortality occurred with 1,306 µm limestone. Hu et al. (2020) reported that coarse limestone (1,062 µm) improved FCR compared to fine limestone (160 µm), but only from d 14 to 20. Other studies have shown no direct effect of limestone particle size on broiler performance (Bradbury et al., 2018; Majeed et al., 2020; Lee et al., 2021; Jafari Arvari et al., 2024), though its influence may depend on factors such as calcium concentration, calcium to P ratio, and calcium source (Bradbury et al., 2018; Hu et al., 2020; Majeed et al., 2020; Lee et al., 2021). Additionally, limestone particle size had no effect on tibia ash (Manangi and Coon, 2007; Majeed et al., 2020; Jafari Arvari et al., 2024). There is a scarcity of studies examining how altering limestone particle size to change its solubility affects broiler growth and health during an enteric challenge. Paiva et al. (2013) observed that a highly soluble calcium source influenced NE severity, suggesting that changes in calcium solubility through limestone particle size may also impact enteric disease severity.

Interactions between calcium concentrations and limestone particle size

Early research by Anderson et al. (1984) demonstrated an interaction between calcium concentration and calcium particle size in broiler nutrition. However, later studies have primarily examined these factors in layer nutrition, particularly their effects on eggshell quality (Zhang and Coon, 1997; de Araujo et al., 2011; Hervo et al., 2022). With growing concerns about excess calcium in broiler diets (Walk, 2016), recent research has focused on the interaction between calcium concentration and limestone particle size and their effects on AID of calcium and P, as well as broiler performance.

Coarse limestone has higher *in vivo* solubility than fine limestone (de Witt et al., 2006), which may help mitigate negative effects of a low-calcium diet (Majeed et al., 2020; Lee et al., 2021). This effect may result from enhanced calcium digestibility (Kim et al., 2018), prolonged calcium release and reduced pH in the gizzard (Lee et al., 2021), and the preservation of phytase efficacy through limited calcium-phytate interactions (Kim et al., 2018). Broilers fed coarse limestone (1,200 µm) and a low calcium diet (6.0 g/kg) had a higher d 21 BW than those fed fine limestone (44 µm) (Lee et al., 2021). Additionally, fine limestone combined with low calcium increased FCR compared to higher calcium levels (Majeed et al., 2020; Lee et al., 2021). However, coarse limestone (500 to 2,000 µm) did not mitigate the negative effects of a low calcium to P ratio on tibia breaking strength and performance in broilers (Hu et al., 2020). While coarse limestone showed similar digestibility to fine limestone (< 500 µm) at low calcium to P ratios, its digestibility was reduced at higher ratios (Hu et al., 2020). Dietary calcium and limestone particle size interactions have also influenced mucosal expression of calcium and P transporters and tight junction proteins in the small intestine (Hu et al., 2022). Some studies have reported no interactions between calcium level and limestone particle size on AID of calcium and P (Anwar et al., 2016; Bradbury et al., 2018), reflecting inconsistent responses. These responses are likely influenced by limestone solubility but also depend on dietary calcium concentrations and phytase levels (Bradbury et al., 2018; Kim et al., 2018; Kim et al., 2019; Majeed et al., 2020; Li et al., 2021).

All previously mentioned broiler studies evaluating calcium concentration and limestone particle size interactions were conducted without an enteric challenge. Research directly addressing this interaction remains limited. Under a naturally occurring NE, Paiva et al. (2013) examined dietary calcium level and calcium source solubility. Although not a direct comparison with limestone particle size, their findings are relevant, as calcium solubility can vary with particle size. The authors reported that higher dietary calcium (0.90 vs. 0.60%) combined with a highly soluble calcium source increased mortality, while the highly soluble calcium source alone negatively affected broiler growth. This may result from higher calcium solubility paired with increased calcium levels, potentially reducing phytate-P hydrolysis (Manangi and Coon, 2007), promoting calcium-phytate complex formation (Selle et al., 2009), and supporting proliferation of *C. perfringens* (Moran, 2014). A paucity of research exists on broiler responses to limestone particle size and calcium concentration, especially during an enteric challenge. Further research is needed to examine how this interaction influences broiler growth and health under enteric disease conditions.

SUMMARY

Optimizing broiler growth, nutrient utilization, and intestinal health is critical for sustainable poultry production, especially as the industry shifts away from the use of AGP. Strategies to reduce reliance on AGP have focused on utilizing feed additives and modifying dietary components. Phytase and butyrate-based feed additives are explored as potential AGP alternatives due to their ability to enhance nutrient availability and modulate intestinal physiology. Phytase degrades phytate, increasing P digestibility and reducing its anti-nutritional effects, whereas CB supports intestinal integrity, pathogen control, and nutrient absorption. While phytase is widely supplemented in broiler diets, interactions between phytase, CB, and BMD remain underexplored, particularly their combined effects on broiler performance, nutrient digestibility, and intestinal health.

In addition to feed additives, dietary calcium levels and limestone particle sizes influence broiler performance and disease resilience. Calcium is essential for skeletal development and metabolic functions, but excessive calcium or highly soluble calcium sources may impair nutrient digestibility and exacerbate enteric disease severity. Limestone, the primary calcium source in broiler diets, varies in particle size, which influences solubility, nutrient absorption, and intestinal pH. Interactions between dietary calcium levels and limestone particle size can influence pathogen proliferation and nutrient utilization. However, broiler research on their combined effects, particularly under enteric challenge conditions, remains limited.

This dissertation includes two studies that examined how feed additives and dietary manipulation influence broiler performance, nutrient utilization, and intestinal health. The first study explored the effects of different phytase concentrations combined with CB and BMD on broiler performance, processing yields, and nutrient digestibility (Part 1), as well as intestinal permeability, jejunal histomorphometry, tight junction gene expression, and cecal microbiome diversity and function (Part 2). The second study investigated the combined impact of limestone particle size and calcium concentration on broiler performance, tibia mineralization, and nutrient digestibility under an enteric challenge with *Eimeria* spp. and *C. perfringens*. Understanding these interactions is critical for refining feeding strategies that optimize broiler growth while mitigating disease challenges.

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III. RESPONSE OF YPM X ROSS 708 MALE BROILERS TO DIETS CONTAINING VARYING INCLUSIONS OF PHYTASE, CALCIUM BUTYRATE, AND BACITRACIN METHYLENE DISALICYLATE FROM 1 TO 42 DAYS OF AGE–PART 1: PERFORMANCE, PROCESSING YIELDS, AND NUTRIENT DIGESTIBILITY

ABSTRACT

This 42-d study evaluated the effects of phytase, calcium butyrate (**CB**), and bacitracin methylene disalicylate 50 (**BMD**) on broiler performance, processing yields, and nutrient digestibility. Ross YPM x 708 male broilers (2,880 total) were distributed in 72 floor pens and assigned to 1 of 9 treatments (8 replicates/treatment) on d of hatch. This experiment was a 2 x 4 + 1 factorial arrangement, including 2 phytase concentrations (500 or 1,500 FTU/kg), 4 microbiota modulating feed additive groups (**MMFA**; none, CB (0.5 g/kg of diet), BMD (55 mg/kg of diet), or both CB and BMD), and a negative control without feed additives. Broiler performance (d 14, 28, and 42), apparent ileal nutrient digestibility (d 28 and 42), and processing yields (d 43) were determined. Day 14 BW increased with BMD inclusion compared to CB and no MMFA in the 1,500 FTU/kg group but BW were similar between all MMFA combined with 500 FTU/kg ($P \leq 0.05$). Supplementing BMD increased d 28 BW and reduced d 1 to 28 feed conversion ratio compared to CB and no MMFA (main effect, $P \leq 0.05$). Day 42 BW varied depending on dietary phytase concentrations. When diets contained 500 FTU/kg, broilers fed both CB and BMD had a higher BW than broilers fed only CB. Whereas when the inclusion of phytase was increased to 1,500 FTU/kg, broilers fed diets with only BMD or both CB and BMD had higher BW than broilers fed diets with no MMFA ($P \leq$

0.05). Phytase concentrations at 1,500 FTU/kg increased ($P \leq 0.05$) digestibility of fat (main effect, d 42), phosphorus (d 28 and 42), and apparent ileal digestible energy (main effect, d 42) compared to 500 FTU/kg. In this study, dietary BMD improved broiler growth compared to CB and no MMFA. However, these observed differences between CB and BMD were dependent on dietary phytase concentrations.

INTRODUCTION

Exogenous phytase is routinely used in broiler diets due to its ability to breakdown phytate (Selle et al., 2000; Selle and Ravindran, 2007; Dersjant-Li et al., 2015; Selle et al., 2023). Phytase can increase broiler performance and nutrient utilization through the liberation of chelated nutrients from phytate and reduction of its anti-nutritional properties (Nelson et al., 1968; Nelson et al., 1971; Perney et al., 1993; Liu et al., 2009; Ptak et al., 2015; Ajuwon et al., 2020). Although phytate is fundamentally an anti-nutrient, it has the beneficial characteristic of contributing to broiler phosphorus requirements, thereby reducing the need for dietary inorganic phosphorus sources (Ibrahim et al., 1999). Over the years, exogenous phytase has been a critically important feed additive to increase broiler growth and feed efficiency. Concurrently, removal or limited use of antibiotic growth promoters (**AGP**) in the European Union and U.S. poultry industries has emphasized feeding strategies that support gastrointestinal health (Roth et al., 2019). A traditionally used AGP in poultry diets is bacitracin methylene disalicylate (**BMD**) which targets gram-positive bacteria such as *Streptococci*, *Staphylococci*, *Clostridia*, *Fusobacterium*, and *Actinomyces* (Proctor and Phillips, 2019). Usage of AGP have increased growth rate, lowered feed conversion ratio (**FCR**), and elevated health of birds through modulation of intestinal microbiota and disease prevention (Engberg et al., 2000; Miles et al., 2006; Mehdi et al., 2018; Maria Cardinal et al., 2019). Removal of these AGP has pressured the poultry industry to establish dietary alternatives to maintain broiler performance during disease challenges.

Possible alternative feeding strategies to AGP usage includes the supplementation of butyric acid (**BA**) or butyrate-based feed additives (Ayalew et al., 2022). Reviews conducted by Guilloteau et al. (2010), Deepa et al. (2018), and El-Saadony et al. (2022) highlighted that endogenous production or dietary supplementation of BA or butyrate can improve pathogen control, promote growth of beneficial bacteria, decrease luminal pH, increase nutrient digestibility and growth, and reduce oxidative stress. Furthermore, research groups indicate that dietary supplementation of BA or butyrate-based feed additives can increase broiler performance (Levy et al., 2015; Kaczmarek et al., 2016; Raza et al., 2019; Sizmaz et al., 2022), nutrient digestibility (Kaczmarek et al., 2016; Liu et al., 2017; Nari and Ghasemi, 2020), and intestinal health (Kaczmarek et al., 2016; Song et al., 2017; Mátis et al., 2022). Dietary usage of BA or butyrate products have been assessed as promising alternatives to AGP (Deepa et al., 2018; Giacomini et al., 2022).

Combining organic acids and phytase as an alternative feeding strategy to AGP could increase broiler growth and nutrient utilization (Vieira et al., 2017; Vieira et al., 2018). An organic acid’s ability to lower digestive tract pH (Esmaeilipour et al., 2011; Han et al., 2023) could limit calcium-phytate formation and optimize phytase activity (Selle et al., 2009). More specifically, butyrate and phytase combinations have potential to positively alter intestinal morphology by increasing villus height and reducing crypt depth (Layter et al., 2021) which could translate to increased performance and feed efficiency (Kaczmarek et al., 2016). Knowledge gaps exist for determining which phytase concentration is more appropriate in combination with other feed additives. Therefore, understanding how broilers respond to organic acids and AGP in combination with exogenous phytase is relevant for poultry producers. This study was conducted to assess the effect of calcium butyrate (**CB**) and BMD combined with varying phytase concentrations on performance, processing yields, and nutrient digestibility of YPM x Ross 708 male broilers during a 42-d grow-out.

MATERIALS AND METHODS

Animal Care

This experiment was conducted at the Auburn University Charles C. Miller, Jr. Poultry Research and Education Center. All procedures involving live birds were approved by the Auburn University Institutional Animal Care and Use Committee (PRN 2022-4061).

Bird Management

A total of 2,880 male d-old broiler chicks (YPM x Ross 708; Aviagen North America, Huntsville, AL) were obtained from a commercial hatchery. All chicks received a coccidiosis vaccine (Coccivac-B52, Merck Animal Health, Madison, NJ) containing live oocysts of *Eimeria* spp. (*E. acervulina*, *E. maxima*, *E. maxima MFP*, *E. mivati*, and *E. tenella*) at the hatchery. Upon arrival, chicks were weighed and distributed randomly among 72 floor pens (40 birds/pen, 0.07 m^2/bird from d 1 to 27; 33 birds/pen, 0.08 m^2/bird from d 28 to 42) in an environmentally controlled house equipped with exhaust fans, stir fans, forced-air heaters, evaporative-cooling pads, and an electronic control panel to manage temperature and ventilation. All floor pens contained used litter from 1 previous flock that was not subjected to a disease challenge. Pens were top-dressed with new pine shavings, contained nipple drinkers (5 nipples/pen), and a hanging pan feeder. Feed and water were provided for *ad libitum* consumption. The lighting program consisted of 24L:0D from 1 to 7 d, 21L:3D from 7 to 10 d, and 20L:4D from 10 to 42 d. Environmental temperature was maintained at 33°C on d 1 and was gradually reduced to 18.3°C by 42 d of age.

Feed Formulation, Manufacture, and Experimental Design

All diets were manufactured at the Auburn University Poultry and Animal Nutrition Center. For all feeding phases, the negative control (**NC**) was formulated following Aviagen recommendations for Ross 708 male broilers (Aviagen, 2019) (**Table 3.1**). However, amino acid (**AA**) density was formulated at 96% of the recommendations, while both calcium and available phosphorus were reduced by 0.20 percentage points compared to their recommended values. A lower AA density was used to account for longer starter, grower, and finisher phases compared to Ross nutrition recommendations (Aviagen, 2019). The NC did not contain phytase (0 FTU/kg) or other feed additives.

Broilers were fed a 3-phase feeding program consisting of starter (d 1 to 14; crumbles), grower (d 15 to 28; pellets), and finisher (d 29 to 42; pellets) diets and randomly assigned to 1 of 9 treatments with 8 replicates per treatment. This experiment was a 2 x 4 + 1 factorial arrangement including 2 concentrations of phytase and 4 groups of microbiota modulating feed additives (**MMFA**). Phytase was included at either 500 or 1,500 FTU/kg (Optiphos Plus, Huvepharma Inc., Peachtree City, GA). Microbiota modulating feed additives included the individual or combined supplementation of CB (ButiPEARL, Kemin Industries Inc., Des Moines, IA) and BMD (BMD 50, Zoetis, Parsippany, NJ). Therefore, the 4 groups of MMFA included no additive, CB (0.5 g supplement/kg of diet), BMD (55 mg/kg of diet), or both CB and BMD. The NC included no experimental feed additives. The 8 treatments with varying inclusion levels of phytase and MMFA were manufactured using the NC diet composition. All 8 treatments contained exogenous phytase. Enzyme activity of the phytase product was 10,000 FTU/g and was expected to release 0.20% calcium and available phosphorus. During formulation, MMFA were added “on-top” of the complete diet without consideration of changes in dietary nutrient content. According to the manufacturer, the encapsulated butyrate product contained 50% CB (0.5 g supplement/kg of diet = 0.25 g CB/kg of diet). As for BMD, 454 g of product was equivalent to 50 g of bacitracin as indicated by the manufacturer.

Whole corn was ground with a hammermill (Model 11.5 × 38, Roskamp Champion, Waterloo, IA) equipped with a 4.76-mm screen. All feed additives (i.e., phytase, CB, and BMD) were premixed with 4.54 kg of ground corn using a countertop mixer (Model A-200, The Hobart Mfg. Co., Troy, OH), prior to their addition to the whole batch of feed. Feed ingredients were blended for 150 s (30 s dry cycle and 120 s wet cycle) using a twin shaft mixer (Model 726, Scott Equipment Co., New Prague, MN). Diets were conditioned at 77°C for 40 s and pelleted through a 4.0-mm pellet die with an effective thickness of 36-mm (length/diameter ratio = 9) using a pellet mill (Model 1112-4, California Pellet Mill Co., Crawfordsville, IN). Pellets were dried and cooled using a counter-flow pellet cooler (Model CC0909, California Pellet Mill Co., Crawfordsville, IN). Starter feed was crumbled in a crumbler with manual roll adjustment (Model 624SS, California Pellet Mill Co., Crawfordsville, IN). During feed manufacture, feed samples were collected at evenly spaced intervals, and mixed to form a composite sample for subsequent nutrient analysis. Crude protein (AOAC official method 990.03; AOAC International, 2006), crude fat (AOCS Standard Procedure Am 5-04; AOCS, 1996), and phosphorus (modified procedure using Wolf et al. (2003) and CEM Application Notes for Acid Digestion (CEM Corporation, 2024) were analyzed by a commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY). Gross energy (**GE**) of feed was determined by the Central Analytical Lab and Water Quality Lab (Fayetteville, AR). Nutrient analysis of feed was used for determination of ileal nutrient digestibility. In addition, feed samples were sent to an external laboratory (BIOVET laboratory, Huvepharma Inc., Peshtera, Bulgaria) for phytase analysis according to the ISO 30024 (2009) procedure, where 1 phytase unit is the amount of enzyme that releases 1 µmol of inorganic orthophosphate from a sodium phytate substrate per min at pH 5.5 and 37°C.

Measurements

Broilers and feed were weighed on d 1 (average chick weight = 37 g), 14, 28, and 42 to determine BW, feed intake (**FI**), and FCR. Birds were inspected twice daily with room temperature, bird condition, mortality, and availability of feed and water monitored during each inspection. The BW of mortalities were used to adjust FCR.

An indigestible marker (0.50% titanium dioxide, **TiO_2**) was added to the grower (15 to 28 d of age) and finisher feed (29 to 42 d of age) for determination of apparent ileal nutrient digestibility. On d 28 and 42, 5 birds per pen were selected randomly and euthanized by CO_2 asphyxiation and cervical dislocation in accordance with American Veterinary Medical Association Guidelines (AVMA, 2020) to collect ileal digesta and evaluate digestibility of protein, fat, phosphorus, and energy. Ileal digesta was collected starting 2 cm posterior from the Meckel’s diverticulum to 2 cm anterior from the ileal-cecal junction by gently removing the ileal contents to provide sufficient sample (> 10 g freeze dried) for nutrient digestibility analysis. While removing the ileal content, distilled water was used to aid in digesta collection. Ileal contents were pooled within individual pens. After collection, pooled ileal digesta samples were stored on ice, transported to the laboratory, and frozen at -20°C until analysis. Upon analysis, ileal digesta samples were lyophilized in a Virtis Genesis Pilot Lyophilizer (SP Industries, Warminster, PA) and then ground using an electric coffee grinder (Capresso 560.4 Infinity, Montvale, NJ) on the finest setting. Thereafter, ileal digesta samples were sent to a commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY) to be analyzed for CP, crude fat, and phosphorus using methods mentioned previously. Duplicate 0.75 g samples of feed and ileal digesta were analyzed by the Central Analytical Lab and Water Quality Lab (Fayetteville, AR) for GE using an isoperibol oxygen bomb calorimeter (model no. 6200, Parr Instruments, Moline, IA) standardized with benzoic acid. Titanium dioxide concentration of feed and ileal digesta was determined through procedures established by Short et al. (1996). Dried feed (600 mg in duplicate) and ileal digesta (200 mg in duplicate) samples were ashed at 580°C and dissolved in sulfuric acid at 250°C. A yellow/orange color was produced after addition of hydrogen peroxide (30%). Color intensity of the solution correlated to TiO_2 concentration of the sample. For each sample, duplicate 1 mL aliquots of the final solution was analyzed with a UV/Vis spectrophotometer (SpectraMax Plus 384 Absorbance Microplate Reader, Molecular Devices, LLC., San Jose, CA) with absorbance measured at 410 nm. Analyzed TiO_2 concentrations (DM basis) for grower feed samples ranged from 0.50 to 0.56% (average CV and recovery for all grower treatments was 3.43 and 92.5%, respectively), whereas finisher feed samples ranged from 0.53 to 0.56% (average CV and recovery for all finisher treatments was 2.08 and 93.7%, respectively). Average TiO_2 recovery was determined using the calculated feed concentration of TiO_2 (DM basis) which was 0.58%. As for ileal digesta samples, d 28 and 42 CV for TiO_2 recovery for all treatments ranged from 2.67 to 9.36% (d 28 average = 5.98%) and 5.14 to 7.95% (d 42 average = 6.26%), respectively. Apparent ileal digestibility (**AID**) of protein, fat, phosphorus, and energy were determined using an equation adapted from Stein et al. (2007):

$$\text{AID \%} = \left\{ \frac{\left[\left(\frac{\text{Nutrient}}{\text{TiO}_2} \right)_{\text{diet}} - \left(\frac{\text{Nutrient}}{\text{TiO}_2} \right)_{\text{digesta}} \right]}{\left(\frac{\text{Nutrient}}{\text{TiO}_2} \right)_{\text{diet}}} \right\} \text{ x } 100$$

where $\left(\frac{\text{Nutrient}}{\text{TiO}_2} \right)$ represents the ratio of protein, fat, phosphorus, or energy to TiO_2 in the diet or ileal digesta. Percent GE digestibility values obtained from this equation were multiplied by the GE content of feed to determine apparent ileal digestible energy (**AIDE**; DM basis) (Gautier and Rochell, 2020). In addition to the 5 birds used to determine apparent ileal nutrient digestibility, 2 birds per pen were euthanized on d 27 for health and physiology measurements (see Chapter IV; Gulizia et al., 2025), bringing the number of birds per pen from 40 to 33.

At 43 d of age, 10 birds per pen were selected randomly, wing banded, marked with a food-grade dye, and processed for the determination of carcass characteristics. Feed was removed from each pen 12 h prior to processing. However, all birds had *ad libitum* access to drinking water during the feed removal period. The selected birds were placed in coops and transported to the Auburn University Fortenberry Processing Plant. Birds were shackled, stunned electrically, exsanguinated, scalded, plucked, and eviscerated manually. After processing, carcasses were static chilled in slush ice (4°C) for 3 h before chilled carcass weights were determined. After chilling, carcasses were drip dried for 3 min and weighed for determination of carcass weights and yields. Carcasses were stored at 4°C for 16 h. Afterwards, all carcasses were deboned utilizing stationary cones to determine total breast fillet (*Pectoralis major*), tenders (*Pectoralis minor*), wings, and legs weight and their corresponding yields. A whole wing included the wing tip, wing portion, and drumette, whereas a leg included the drumstick and thigh. Carcass yields were calculated relative to 43 d live weight, while breast, tender, wing, and leg yields were calculated relative to chilled carcass weight.

Statistical Analyses

This experiment was a completely randomized design with data analyzed as a 2 x 4 factorial to evaluate the main effects of phytase and MMFA and their interactions. There were 2 concentrations of phytase (500 or 1,500 FTU/kg) and 4 groups of MMFA (none, only CB, only BMD, or both CB and BMD). Excluded from this factorial analysis was the NC treatment. Instead, independent orthogonal contrasts were performed for comparison between phytase supplemented diets with no MMFA (NC + 500 FTU/kg and NC + 1,500 FTU/kg) and the NC (0 FTU/kg). In addition, orthogonal polynomial contrasts were performed for the unequally spaced phytase concentrations (0, 500, and 1,500 FTU/kg). The ORPOL function in PROC IML was used for determination of CONTRAST coefficients for the unequally spaced treatments.

Each treatment was represented by 8 replicate pens with pen considered as the experimental unit. Percentage data were arcsine transformed before statistical analysis. Using Tukey's method, outliers were determined when values were 1.5 times the interquartile range below and above the first and third quartile, respectively (Tukey, 1977). All data were analyzed using the GLIMMIX procedure of the SAS software version 9.4 (SAS Institute Inc., Cary, NC). Least square means were compared using Tukey-Kramer with statistical significance considered at $P \leq 0.05$. For all means, 95% confidence limits were determined.

RESULTS

Feed and Phytase Analysis

Analysis of DM, GE, CP, total phosphorus, and crude fat for the NC fed during the starter, grower, and finisher phases are shown in **Table 3.1**. Nutrient analyses were averaged between all treatments manufactured using the NC. Negative control diets devoid of exogenous phytase had less than 100 FTU/kg of activity for all feeding phases. Treatments calculated for 500 FTU/kg had an average phytase concentration of 406 (SD = 54 FTU/kg; CV = 13.34%; Recovery = 81.3%), 506 (SD = 37 FTU/kg; CV = 7.27%; Recovery = 101.1%), and 408 FTU/kg (SD = 36 FTU/kg; CV = 8.75%; Recovery = 81.6%) for the starter, grower, and finisher diets, respectively. As for the 1,500 FTU/kg treatments, phytase averaged 934 (SD = 71 FTU/kg; CV = 7.57%; Recovery = 62.3%), 921 (SD = 50 FTU/kg; CV = 5.41%; Recovery = 61.4%), and 944 FTU/kg (SD = 35 FTU/kg; CV = 3.76%; Recovery = 63.0%) for the starter, grower, and finisher diets, respectively. All feed samples were stored for an extended period before lab analysis, which could have negatively affected the analyzed phytase values, particularly in diets containing 1,500 FTU/kg.

Broiler Performance and Mortality

There were multiple broiler performance differences between the NC (0 FTU/kg) and NC with phytase inclusion (500 and 1,500 FTU/kg) as indicated by the independent orthogonal and orthogonal polynomial contrasts (**Table 3.2**). Broilers consuming the NC + 500 FTU/kg of phytase had a higher BW (d 14, 28, and 42) and FI (d 1 to 28 and 1 to 42) with a lower FCR (d 1 to 14) compared to the NC ($P \leq 0.05$). Increasing phytase supplementation to 1,500 FTU/kg in the NC increased BW (d 14) and lowered FCR (d 1 to 14 and 1 to 28) compared to the NC ($P \leq 0.05$). A linear response to increasing phytase concentrations was observed for d 1 to 14 and 1 to 28 FCR ($P \leq 0.05$). As phytase concentration increased, there was a corresponding decrease in FCR. In addition, quadratic responses to increasing phytase concentrations were observed for BW (d 14, 28, and 42) and d 1 to 42 FI ($P \leq 0.05$), with the highest values observed for birds fed 500 FTU/kg.

The influence of phytase and MMFA on broiler performance is shown in **Table 3.3**. Independent of MMFA, only d 1 to 42 FCR was influenced by phytase concentration. Broilers fed diets with 1,500 FTU/kg had lower FCR compared to 500 FTU/kg ($P \leq 0.05$). Supplementing only BMD or both CB and BMD increased broiler BW on d 28 and reduced d 1 to 28 FCR compared to no MMFA and only CB ($P \leq 0.05$). Day 1 to 28 FI was higher when broilers were fed diets containing both CB and BMD compared to broilers fed diets with only CB ($P \leq 0.05$). Feed conversion ratio from d 1 to 14 was lower when broilers consumed a diet with only BMD or both CB and BMD compared to broilers fed diets with only CB ($P \leq 0.05$). In addition, dietary supplementation of only BMD lowered d 1 to 14 FCR compared to no MMFA ($P \leq 0.05$).

Interactions between phytase and MMFA were observed for BW and FI ($P \leq 0.05$). Day 14 BW was not influenced by MMFA within the 500 FTU/kg group. However, when the inclusion of phytase was 1,500 FTU/kg, broilers fed diets with BMD either with or without CB had higher BW ($P \leq 0.05$) compared to broilers fed diets without MMFA or only with CB. Within the 500 FTU/kg group, broilers fed a diet with both CB and BMD had a higher d 42 BW compared to only CB. When the inclusion of phytase was increased to 1,500 FTU/kg, broiler fed diets with only BMD or both CB and BMD had higher d 42 BW compared to broilers fed diets with no MMFA ($P \leq 0.05$). At a phytase concentration of 500 FTU/kg, d 1 to 14 FI across treatments showed minimal variation. However, with an increase in phytase concentration to 1,500 FTU/kg, the inclusion of both CB and BMD led to a 24 g increase in FI compared to broilers fed diets without MMFA ($P \leq 0.05$). This interaction was described by determining the overlap of 95% confidence limits between treatments (Cumming and Fidler, 2009), since the Tukey-Kramer test was unable to separate the means.

Independent orthogonal and orthogonal polynomial contrasts between the NC (0 FTU/kg) and NC with phytase inclusion (500 and 1,500 FTU/kg) showed no influence of phytase on broiler mortality ($P > 0.05$) (**Table 3.4**). Within the factorial, phytase and MMFA did not influence broiler mortality during the entire trial ($P > 0.05$) (**Table 3.5**).

Processing Yields

Processing yields were not different between the NC and NC supplemented with phytase ($P > 0.05$) (**Table 3.6**). Additionally, no linear or quadratic responses to increasing phytase concentrations were observed for processing yields ($P > 0.05$).

Supplementation of phytase and MMFA did not influence processing yields ($P > 0.05$) (**Table 3.7**).

Nutrient Digestibility

Independent orthogonal and orthogonal polynomial contrast were conducted to evaluate the effect of phytase concentration on d 28 nutrient digestibility (**Table 3.8**). Broilers fed NC + 500 FTU/kg diets had higher digestibility of fat and phosphorus and AIDE compared to broilers fed the NC without phytase ($P \leq 0.05$). In addition, broilers consuming NC + 1,500 FTU/kg had a higher phosphorus digestibility compared to broilers fed the NC without phytase ($P \leq 0.05$). Orthogonal polynomial contrasts revealed a quadratic response to increasing phytase concentrations for phosphorous digestibility and AIDE on d 28 ($P \leq 0.05$). Increasing phytase concentrations from 0 to 500 FTU/kg (15.49% difference) yielded a higher magnitude increase in phosphorus digestibility compared to the increase from 500 to 1,500 FTU/kg (10.40% difference). As for AIDE, the highest value was observed at 500 FTU/kg.

Day 28 nutrient digestibility is shown in **Table 3.9**. Broilers fed diets with only BMD and CB and BMD had higher ($P \leq 0.05$) d 28 CP digestibility compared to birds fed diets without MMFA. Furthermore, dietary inclusion of both CB and BMD increased d 28 CP digestibility compared to only CB ($P \leq 0.05$). There was an interaction between phytase and MMFA on phosphorus digestibility and AIDE on d 28 ($P \leq 0.05$). Day 28 phosphorus digestibility was similar between all MMFA within the 500 and 1,500 FTU/kg groups. Broilers had a higher d 28 phosphorus digestibility when fed a diet with 1,500 FTU/kg of phytase compared to 500 FTU/kg ($P \leq 0.05$). Additionally, when phytase inclusion was at 500 FTU/kg, the inclusion of CB or BMD alone or in combination did not change AIDE compared to a diet without MMFA. However, at the higher phytase inclusion level of 1,500 FTU/kg, broilers consuming diets with only BMD showed higher AIDE compared to those on a diet with no MMFA ($P \leq 0.05$).

As indicated by the independent orthogonal contrasts, only d 42 phosphorus digestibility was increased with NC + 500 FTU/kg of phytase compared to the NC ($P \leq 0.05$) (**Table 3.10**). As for NC + 1,500 FTU/kg, d 42 digestibility of CP and phosphorus and AIDE was higher compared to the NC ($P \leq 0.05$). Orthogonal polynomial contrasts indicated linear (d 42 CP digestibility and AIDE) and quadratic (d 42 phosphorus digestibility) responses to increasing phytase concentrations ($P \leq 0.05$). Day 42 CP digestibility and AIDE increased when increasing phytase concentrations from 0 to 1,500 FTU/kg. Increasing phytase concentrations from 0 to 500 FTU/kg (22.97% difference) resulted in a higher magnitude increase in d 42 phosphorus digestibility compared to 500 to 1,500 FTU/kg (13.44% difference).

Day 42 nutrient digestibility is shown in **Table 3.11**. Broilers fed diets with 1,500 FTU/kg of phytase had higher ($P \leq 0.05$) fat digestibility and AIDE compared to broilers fed diets with 500 FTU/kg of phytase. There was a phytase and MMFA interaction ($P \leq 0.05$) for phosphorus digestibility. Day 42 phosphorus digestibility increased when broilers were fed diets supplemented only BMD compared to broilers fed diets supplemented with only CB in the 500 FTU/kg group. However, supplementation of MMFA did not influence d 42 phosphorus digestibility in the 1,500 FTU/kg group.

DISCUSSION

The objective of this experiment was to determine the effects of CB and BMD combined with varying phytase concentrations on performance, processing yields, and nutrient digestibility of male broilers reared to 42 d of age. Depending on the concentration (500 or 1,500 FTU/kg), phytase increased broiler growth and nutrient digestibility compared with the NC (0 FTU/kg) in the present study. This agrees with previous findings that demonstrated the effectiveness of dietary phytase supplementation in increasing broiler BW, reducing FCR, and elevating nutrient digestibility compared to diets without phytase (Santos et al., 2008; Liu et al., 2010; Walters et al., 2019). In the present study, there were minimal differences in broiler performance and nutrient digestibility (grower phase) when dietary phytase concentrations were increased from 500 to 1,500 FTU/kg. In the grower phase (15 to 28 d of age), phosphorus digestibility was increased with 1,500 FTU/kg of phytase, but no other differences in nutrient digestibility were observed. The lack of differences in nutrient digestibility during the early growth phases may explain why no differences in live performance were observed between broilers fed diets with either 500 or 1,500 FTU/kg of phytase. It should also be noted that phosphorus digestibility values in the present study may be underestimated, as digesta was collected from the entire ileum. Research by Rodehutscord et al. (2012) has shown that phosphorus digestibility is lower in the proximal ileum compared to the medial and distal portions. In the finisher phase (29 to 42 d of age), digestibility of fat and phosphorus and AIDE were increased with 1,500 FTU/kg of phytase. Phytase inclusion can increase fat (Liu et al., 2010), phosphorus (Manobhavan et al., 2016), and energy digestibility (Leyva-Jimenez et al., 2019) in broilers. An increase in fat and energy digestibility via exogenous phytase can be related to multiple reasons. These include phytase's ability to reduce the anti-nutritional factor of phytate, limit or reduce endogenous losses, and by subsequently releasing phytate bound nutrients (Ravindran et al., 2000; Camden et al., 2001; Santos et al., 2008; Selle et al., 2009; Walters et al., 2019). In addition, phytase can increase CP (Santos et al., 2008; Liu et al., 2009) and AA digestibility (Gehring et al., 2013; Walters et al., 2019), which can lead to increased muscle deposition. Regardless of the mechanism, 1,500 FTU/kg of phytase has been shown to increase breast meat yield compared to 500 FTU/kg (Smith et al., 2019). However, this effect was not observed in the present study or in previous research conducted in our lab (Gulizia et al., 2023).

In the present study, CB, BMD, and their combination were defined as microbiota modulating feed additives. The current results showed that dietary inclusion of BMD at 55 ppm increased broiler BW (d 14, 28, and 42) and reduced FCR (d 1 to 14 and 1 to 28). Other researchers have observed increased broiler performance with the supplementation of AGP compared with no AGP (Murugesan et al., 2015; Manafi et al., 2017; Li et al., 2022) and butyrate (Naghizadeh et al., 2022a; Naghizadeh et al., 2022b). Supplementation of BMD had a positive effect on broiler FCR during the early growth periods. However, by the conclusion of the study, broilers fed no MMFA, CB, BMD, or both CB and BMD had similar cumulative FCR (d 1 to 42). The effects of AGP on broiler processing yields are varied in the literature with some observing higher dressing percentage and percentage yield (Izat et al., 1990) and others observing no responses (Leeson et al., 2005; Attia et al., 2016). A positive BMD effect on processing yields was not observed in this study even though BMD increased d 28 protein digestibility compared to no AGP inclusion. Similarly, Murugesan et al. (2015) and Manafi et al. (2019) observed an increase in CP digestibility when broilers received BMD compared with those without AGP inclusion. Increased CP digestibility in groups receiving BMD could be attributed to a positive modulation of the gut microbiota and improvements in intestinal morphology (Murugesan et al., 2014; Murugesan et al., 2015; Kumar et al., 2019). Bacitracin modulates the gut microbiota by inhibiting bacterial cell wall synthesis (Smith and Weinberg, 1962). Additionally, the increased CP digestibility resulting from BMD use may reduce the availability of excess protein and amino acids, which could otherwise promote proliferation of pathogens and toxins in the post-ileal digestive tract (Apajalahti and Vienola, 2016).

Butyrate, a product from microbial fermentation of carbohydrates, is a short chain fatty acid that is a natural substance found in the gastrointestinal tract (Guilloteau et al., 2010; Levy et al., 2015). Previous research demonstrated that dietary supplementation of BA or butyrate products either increased broiler performance (Levy et al., 2015; Kaczmarek et al., 2016; Raza et al., 2019) or resulted in similar performance compared with those fed diets with an AGP (Leeson et al., 2005; Kaczmarek et al., 2016; Sikandar et al., 2017). Other researchers have reported no influence of BA or butyrate inclusion on broiler performance (Adhikari et al., 2023), processing weights (Raza et al., 2019), or processing yields (Leeson et al., 2005). In contrast, dietary BA or butyrate supplementation has altered processing characteristics by increasing carcass and breast yield (Dehghani-Tafti and Jahanian, 2016; Adhikari et al., 2023) while also reducing abdominal fat (Panda et al., 2009). Additionally, butyrate supplementation could increase CP, fat, and energy digestibility (Kaczmarek et al., 2016; Liu et al., 2017; Nari and Ghasemi, 2020). However, in the current study, CB supplementation did not increase broiler processing characteristics or nutrient digestibility when compared with those fed the no MMFA or BMD treatments. Higher broiler performance and nutrient digestibility with dietary BA or butyrate supplementation have been attributed to positive modulation of intestinal microbiota (Panda et al., 2009; Wu et al., 2018), increased mineral solubility and absorption (Adil et al., 2010), and elevated nutrient absorption capacity by altering intestinal morphology (Sikandar et al., 2017; Raza et al., 2019). Although butyrate has been reported to alter the intestinal microbiota (Wan et al., 2022), Naghizadeh et al. (2022a) reported no differences in bacterial counts (e.g., *Enterococci, Clostridium perfringens,* and *Lactobacillus salivarius*) when broilers consumed a diet with no butyrate compared with those fed a diet with butyrate. In the present study, CB had minimal to no influence on performance and nutrient digestibility which could indicate that these previously observed effects are variable.

Interactions between phytase and MMFA could impact performance and nutrient digestibility. A review by Vieira et al. (2017) indicated that combining citric acid and phytase in broiler feed could increase weight gain and bone mineralization. Past research has focused on interactions between citric acid and phytase in broiler feeding compared to other organic acids (Vieira et al., 2018). A possible reason for the greater number of research trials using citric acid over BA is its gastrointestinal pH reducing potential. Gastrointestinal pH reductions are dependent on the organic acid's pKa (Vieira et al., 2018). Compared to citric acid, BA has a higher pKa, which could limit its ability to alter gastric pH (Vieira et al., 2018). Successfully reducing gastric pH can result in potential antimicrobial effects (Biggs and Parsons, 2008) and a more favorable environment for phytase activity (Vieira et al., 2018). Consequently, it is interesting to evaluate CB and phytase interactions. Combinations of CB and phytase can lead to similar broiler growth and nutrient digestibility measurements compared with an AGP. As observed with significant phytase and MMFA interactions, broilers fed a diet with CB had a similar BW (d 14 and 42), phosphorus digestibility (d 28 and 42), and AIDE (d 28) as those fed a diet with BMD. However, these responses were dependent on phytase concentrations. Supplementing either CB or BMD alone with 500 FTU/kg of phytase produced broilers with similar d 14 BW, whereas combining those MMFA with 1,500 FTU/kg led to differences in growth. An opposite response was observed for d 42 phosphorus digestibility. Phytase inclusion of 500 FTU/kg combined with either CB or BMD led to differences in phosphorus digestibility, but not when combined with 1,500 FTU/kg. Therefore, depending on concentrations, combining CB and phytase could be a viable alternative broiler feeding strategy when compared to BMD. However, this will depend on rearing conditions and other dietary additives.

In summary, dietary supplementation of BMD (55 ppm) in comparison to CB (0.5 g supplement/kg of diet) and no MMFA increased broiler BW, reduced FCR, and increased CP digestibility (main effect). However, these performance and nutrient digestibility responses to MMFA (CB and BMD) varied depending on dietary phytase concentrations. Of the variables investigated with an observed interaction, 500 and 1,500 FTU/kg of phytase influenced BW and phosphorus digestibility responses to the MMFA groups. With phytase being ubiquitous in broiler feeding, nutritionists should consider the interaction of phytase and additional feed additives when assessing AGP alternatives.

FUNDING SOURCE

This research was funded by a USDA-ARS Cooperative Agreement (G00014614 - Dietary manipulations to improve gut health in broiler production).

ACKNOWLEDGEMENTS

The authors are grateful for the graduate students, undergraduate students, and visiting research scholars from Drs. Pacheco, Hauck, Dozier, and Macklin labs for their assistance during this experiment. Additionally, the authors are grateful for the Auburn University Charles. C. Miller Jr. Research Center and Poultry and Animal Nutrition Center staff for their constant support and contributions during each experiment. Lastly, the authors greatly appreciate Huvepharma Inc. for helping with phytase analysis.

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Table 3.1. Ingredient and nutrient composition of the negative control (NC) with varying concentrations of phytase, calcium butyrate (CB), and bacitracin methylene disalicylate (BMD) fed to YPM x Ross 708 male broilers from 1 to 42 d of age.

	Starter, 1 to 14 d	Grower, 15 to 28 d	Finisher, 29 to 42 d
Ingredient, % as-fed (unless otherwise noted)	NC	NC	NC
Corn	53.81	57.54	61.77
Soybean meal, 46% CP	34.92	30.22	25.52
Distillers dried grains with solubles (DDGS)	5.00	5.00	5.00
Poultry oil	2.77	3.63	4.49
Dicalcium phosphate, 18% P	0.98	0.76	0.55
Calcium carbonate	1.13	1.05	0.98
Salt	0.43	0.43	0.43
DL-Methionine	0.33	0.30	0.26
L-Lysine	0.22	0.20	0.19
L-Threonine	0.14	0.11	0.08
Trace mineral premix[1]	0.10	0.10	0.10
Vitamin premix[2]	0.10	0.08	0.05
Choline chloride	0.08	0.08	0.08
Phytase[3], g/kg	0.00	0.00	0.00
CB[4], g/kg	0.00	0.00	0.00
BMD[5], ppm	0.00	0.00	0.00
Titanium dioxide (TiO_2)	–	0.50	0.50
Calculated nutrients[6], % as-fed (unless otherwise noted)			
DM	83.19	82.61	82.03
AMEn, kcal/kg	3,000	3,100	3,200
CP	22.37	20.36	18.35
Calcium	0.76	0.67	0.58
Available phosphorous	0.28	0.24	0.19
Digestible Lys	1.23	1.10	0.98
Digestible Met + Cys	0.91	0.84	0.77
Digestible Met	0.62	0.57	0.52
Digestible Thr	0.83	0.74	0.65
Digestible Val	0.92	0.84	0.76
Analyzed nutrients[7], % as-fed (unless otherwise noted)			
DM	85.80-86.20	86.30-87.10 (86.57)	85.00-86.20 (85.76)
Gross energy[8], kcal/kg	–[9]	3,854-3,926 (3,879)	3,841-3,961 (3,899)
CP	19.80-22.20	18.19-20.11 (18.92)	16.29-18.53 (17.49)
Total phosphorous	0.53-0.59 (0.57)	0.49-0.53 (0.51)	0.44-0.48 (0.46)
Crude fat	5.56-6.03 (5.80)	6.44-7.05 (6.76)	7.17-7.86 (7.49)

[1] Mineral premix included per kg of diet: Mn (manganese sulfate), 120 mg; Zn (zinc sulfate), 100 mg; Fe (iron sulfate monohydrate), 30 mg; Cu (tri-basic copper chloride), 8 mg; I (ethylenediamine dihydriodide), 1.4 mg; and Se (sodium selenite), 0.3 mg.

[2] Vitamin premix included per kg of diet: Vitamin A (Vitamin A acetate), starter: 18,739 IU, grower: 14,991 IU, finisher: 9,369 IU; Vitamin D (cholecalciferol), starter: 6,614 IU, grower: 5,291 IU, finisher: 3,307 IU; Vitamin E (DL-alpha tocopherol acetate), starter: 66 IU, grower: 53 IU, finisher: 33 IU; menadione (menadione sodium bisulfate complex), starter: 4 mg, grower: 3 mg, finisher: 2 mg; Vitamin B12 (cyanocobalamin), starter: 0.03 mg, grower: 0.03 mg, finisher: 0.02 mg; folacin (folic acid), starter: 2.6 mg, grower: 2.1 mg, finisher: 1.3 mg; D-pantothenic acid (calcium pantothenate), starter: 31 mg, grower: 25 mg, finisher: 15 mg; riboflavin (riboflavin), starter: 22 mg, grower: 18 mg, finisher: 11 mg; niacin (niacinamide), starter: 88 mg, grower: 71 mg, finisher: 44 mg; thiamine (thiamine mononitrate), starter: 5.5 mg, grower: 4.4 mg, finisher: 2.8 mg; D-biotin (biotin), starter: 0.18 mg, grower: 0.14 mg, finisher: 0.09 mg; and pyridoxine (pyridoxine hydrochloride), starter: 7.7 mg, grower: 6.2 mg, finisher: 3.9 mg.

[3] OptiPhos Plus (Huvepharma Inc., Peachtree City, GA) provided 500 FTU/kg (0.05 g/kg) or 1,500 FTU/kg (0.15 g/kg) of phytase activity per kg of diet.

[4] ButiPEARL (Kemin Industries Inc., Des Moines, IA) included at 0 or 0.5 g supplement/kg of diet.

[5] Bacitracin methylene disalicylate 50 Type A Medicated Article (Zoetis, Parsippany, NJ) included at 0 or 55 mg per kg of diet.

[6] Calculated nutrients followed Aviagen recommendations for Ross 708 male broilers (Aviagen, 2019), except for digestible amino acids (96% of the recommendations) and both calcium and available phosphorus (reduced by 0.20 percentage points).

[7] Dry matter, CP, total phosphorus, and crude fat analyses were performed by Dairy One Forage Laboratory (Ithaca, NY); provided are ranges of analyzed nutrients for all treatments which were manufactured using the NC; average analyzed nutrients are in parenthesis.

[8] Gross energy was analyzed at the Central Analytical Lab and Water Quality Lab (Fayetteville, AR).

[9] Gross energy was only analyzed for grower and finisher diets to calculate apparent ileal digestible energy.

Table 3.2. Independent orthogonal and orthogonal polynomial contrasts[1] for performance between YPM x Ross 708 male broilers provided a negative control diet (NC; 0 FTU/kg) and a NC with phytase supplementation (500 or 1,500 FTU/kg) from 1 to 42 d of age[2].

	BW, g/bird			Feed intake, g/bird			Feed conversion ratio, g:g[3]		
	Days of age								
Item	14	28	42	1 to 14	1 to 28	1 to 42	1 to 14	1 to 28	1 to 42
NC vs. NC + phytase[4]									
NC	428	1,571	3,096	495	2,121	4,780	1.149	1.349	1.564
NC + 500 FTU/kg	448	1,611	3,189	501	2,169	4,918	1.125	1.338	1.554
NC + 1,500 FTU/kg	440	1,595	3,141	485	2,151	4,858	1.100	1.335	1.561
CLM[5]	± 7	± 24	± 50	± 10	± 32	± 65	± 0.014	± 0.010	± 0.016
	P-value								
NC vs. NC + 500 FTU/kg	<0.001	0.023	0.009	0.344	0.033	0.002	0.014	0.107	0.372
NC vs. NC + 1,500 FTU/kg	0.013	0.165	0.192	0.159	0.191	0.084	<0.001	0.031	0.794
Linear	0.065	0.311	0.398	0.086	0.329	0.225	<0.001	0.044	0.929
Quadratic	<0.001	0.038	0.011	0.108	0.052	0.004	0.344	0.312	0.360

[1] Orthogonal polynomial contrasts were performed for the unequally spaced phytase concentrations (0, 500, and 1,500 FTU/kg) without MMFA.

[2] Values are least square means of 8 replicate pens, with each pen containing 40 broilers at placement; statistical significance was considered at $P \leq 0.05$.

[3] Feed conversion ratio corrected for mortality.

[4] OptiPhos Plus (Huvepharma Inc., Peachtree City, GA) provided 500 FTU/kg or 1,500 FTU/kg of phytase activity per kg of diet.

[5] CLM = 95% confidence limit for the mean.

Table 3.3. Performance of YPM x Ross 708 male broilers provided a negative control diet varying in phytase, calcium butyrate (CB), and bacitracin methylene disalicylate (BMD) inclusion from 1 to 42 d of age[1].

		BW, g/bird			Feed intake, g/bird			Feed conversion ratio, g:g[2]		
		Days of age								
Item		14	28	42	1 to 14	1 to 28	1 to 42	1 to 14	1 to 28	1 to 42
Phytase[3], FTU/kg, main effect (n = 32)										
500		449	1,628	3,196	498	2,174	4,934	1.107	1.331	1.554^{a}
1,500		452	1,627	3,221	498	2,169	4,901	1.101	1.324	1.538^{b}
CLM[4]		± 4	± 13	± 25	± 5	± 16	± 31	± 0.008	± 0.005	± 0.008
Microbiota modulating feed additives, main effect (n = 16)										
None		444	1,603^{b}	3,165	493	2,160^{ab}	4,888	1.112^{ab}	1.336^{a}	1.557
Only CB[5]		444	1,597^{b}	3,167	500	2,142^{b}	4,890	1.119^{a}	1.338^{a}	1.550
Only BMD[6]		455	1,648^{a}	3,244	497	2,183^{ab}	4,949	1.090^{c}	1.319^{b}	1.541
Both CB and BMD		460	1,662^{a}	3,257	503	2,201^{a}	4,943	1.093^{bc}	1.318^{b}	1.536
CLM		± 6	± 19	± 36	± 8	± 23	± 45	± 0.011	± 0.007	± 0.011
Phytase, FTU/kg x	Microbiota modulating feed additives									
500	None	448^{bc}	1,611	3,189^{ab}	501	2,169	4,918	1.125	1.338	1.554
500	Only CB	446^{bc}	1,597	3,132^{b}	504	2,140	4,877	1.120	1.343	1.563
500	Only BMD	450^{bc}	1,643	3,197^{ab}	492	2,183	4,955	1.088	1.319	1.557
500	Both CB and BMD	453^{abc}	1,661	3,264^{a}	497	2,205	4,984	1.094	1.323	1.542
1,500	None	440^{c}	1,595	3,141^{b}	485	2,151	4,858	1.100	1.335	1.561
1,500	Only CB	442^{c}	1,596	3,203^{ab}	496	2,145	4,902	1.112	1.332	1.537
1,500	Only BMD	459^{ab}	1,653	3,291^{a}	503	2,184	4,942	1.092	1.318	1.526
1,500	Both CB and BMD	468^{a}	1,663	3,250^{a}	509	2,198	4,902	1.092	1.313	1.530
CLM		± 8	± 28	± 50	± 12	± 33	± 67	± 0.016	± 0.010	± 0.016
		P-value								
Phytase		0.211	0.900	0.144	0.887	0.673	0.131	0.256	0.061	0.009
Microbiota modulating feed additives		<0.001	<0.001	<0.001	0.305	0.003	0.080	<0.001	<0.001	0.052
Phytase x Microbiota modulating feed additives		0.012	0.783	0.014	0.020[†]	0.894	0.303	0.187	0.663	0.097

$^{a\text{-}c}$Means within a column with different superscripts differ significantly ($P \leq 0.05$).

[1] Interaction values are least square means of 8 replicate pens, with each pen containing 40 broilers at placement.

[2] Feed conversion ratio corrected for mortality.

[3] OptiPhos Plus (Huvepharma Inc., Peachtree City, GA) provided 500 FTU/kg or 1,500 FTU/kg of phytase activity per kg of diet.

[4] CLM = 95% confidence limit for the mean.

[5] ButiPEARL (Kemin Industries Inc., Des Moines, IA) included at 0 or 0.5 g supplement/kg of diet.

[6] Bacitracin methylene disalicylate 50 Type A Medicated Article (Zoetis, Parsippany, NJ) included at 0 or 55 mg per kg of diet.

[†] Overall interaction was significant; however, Tukey-Kramer multiple comparison test did not separate the means.

Table 3.4. Independent orthogonal and orthogonal polynomial contrasts[1] for mortality between YPM x Ross 708 male broilers provided a negative control diet (NC; 0 FTU/kg) and a NC with phytase supplementation (500 or 1,500 FTU/kg) from 1 to 42 d of age[2].

	Mortality, %		
	Days of age		
Item	1 to 14	1 to 28	1 to 42
NC vs. NC + phytase[3]			
NC	1.56	3.75	4.38
NC + 500 FTU/kg	0.94	3.13	7.19
NC + 1,500 FTU/kg	1.25	3.75	5.31
CLM[4]	± 0.83	± 1.60	± 2.28
		P-value	
NC vs. NC + 500 FTU/kg	0.293	0.912	0.068
NC vs. NC + 1,500 FTU/kg	0.598	0.968	0.506
Linear	0.730	0.985	0.747
Quadratic	0.320	0.912	0.068

[1] Orthogonal polynomial contrasts were performed for the unequally spaced phytase concentrations (0, 500, and 1,500 FTU/kg) without MMFA.

[2] Values are least square means of 8 replicate pens, with each pen containing 40 broilers at placement; statistical significance was considered at $P \leq 0.05$.

[3] OptiPhos Plus (Huvepharma Inc., Peachtree City, GA) provided 500 FTU/kg or 1,500 FTU/kg of phytase activity per kg of diet.

[4] CLM = 95% confidence limit for the mean.

Table 3.5. Mortality of YPM x Ross 708 male broilers provided a negative control diet varying in phytase, calcium butyrate (CB), and bacitracin methylene disalicylate (BMD) inclusion from 1 to 42 d of age[1].

		Mortality, %		
		Days of age		
Item		1 to 14	1 to 28	1 to 42
Phytase[2], FTU/kg, main effect (n = 32)				
500		0.55	2.66	5.70
1,500		0.94	3.13	4.92
CLM[3]		± 0.41	± 0.78	± 1.14
Microbiota modulating feed additives, main effect (n = 16)				
None		1.09	3.44	6.25
Only CB[4]		0.47	2.81	5.16
Only BMD[5]		0.94	3.28	5.31
Both CB and BMD		0.47	2.03	4.53
CLM		± 0.58	± 1.11	± 1.62
Phytase, FTU/kg x	Microbiota modulating feed additives			
500	None	0.94	3.13	7.19
500	Only CB	0.31	2.19	4.06
500	Only BMD	0.63	3.44	6.56
500	Both CB and BMD	0.31	1.88	5.00
1,500	None	1.25	3.75	5.31
1,500	Only CB	0.63	3.44	6.25
1,500	Only BMD	1.25	3.13	4.06
1,500	Both CB and BMD	0.63	2.19	4.06
CLM		± 0.82	± 1.57	± 2.29
			P-value	
Phytase		0.185	0.718	0.296
Microbiota modulating feed additives		0.309	0.369	0.557
Phytase x Microbiota modulating feed additives		0.975	0.601	0.198

[1] Interaction values are least square means of 8 replicate pens, with each pen containing 40 broilers at placement.

[2] OptiPhos Plus (Huvepharma Inc., Peachtree City, GA) provided 500 FTU/kg or 1,500 FTU/kg of phytase activity per kg of diet.

[3] CLM = 95% confidence limit for the mean.

[4] ButiPEARL (Kemin Industries Inc., Des Moines, IA) included at 0 or 0.5 g supplement/kg of diet.

[5] Bacitracin methylene disalicylate 50 Type A Medicated Article (Zoetis, Parsippany, NJ) included at 0 or 55 mg per kg of diet.

Table 3.6. Independent orthogonal and orthogonal polynomial contrasts[1] for carcass and breast meat yields[2] (%) between YPM x Ross 708 male broilers provided a negative control diet (NC; 0 FTU/kg) and a NC with phytase supplementation (500 or 1,500 FTU/kg) at 43 d of age[3].

Item	Chilled carcass	Fillet[4]	Tender[4]	Wing[4]	Leg[4]
NC vs. NC + phytase[5]					
NC	78.04	30.10	6.11	9.74	27.10
NC + 500 FTU/kg	78.08	29.63	6.19	9.74	27.03
NC + 1,500 FTU/kg	77.85	29.62	6.17	9.89	27.16
CLM[6]	± 1.37	± 0.44	± 0.13	± 0.12	± 0.32
			P-value		
NC vs. NC + 500 FTU/kg	0.986	0.145	0.393	0.942	0.760
NC vs. NC + 1,500 FTU/kg	0.810	0.139	0.554	0.074	0.773
Linear	0.795	0.194	0.646	0.053	0.701
Quadratic	0.944	0.269	0.449	0.566	0.648

[1] Orthogonal polynomial contrasts were performed for the unequally spaced phytase concentrations (0, 500, and 1,500 FTU/kg) without MMFA.

[2] Carcass yields were calculated relative to 43 d live weight, while fillet, tender, wing, and leg yields were calculated relative to chilled carcass weight.

[3] Values are least square means of 8 replicate pens, with each pen having 10 broilers processed on d 43; statistical significance was considered at $P \leq 0.05$.

[4] Fillet = combined weight of right and left *Pectoralis major*; Tender = combined weight of right and left *Pectoralis minor*; Wing = the wing tip, wing portion, and drumette; Leg = the drumstick and thigh.

[5] OptiPhos Plus (Huvepharma Inc., Peachtree City, GA) provided 500 FTU/kg or 1,500 FTU/kg of phytase activity per kg of diet.

[6] CLM = 95% confidence limit for the mean.

Table 3.7. Carcass and breast meat yields[1] (%) of YPM x Ross 708 male broilers provided a negative control diet varying in phytase, calcium butyrate (CB), and bacitracin methylene disalicylate (BMD) inclusion at 43 d of age[2].

Item		Chilled carcass	Fillet[3]	Tender[3]	Wing[3]	Leg[3]
Phytase[4], FTU/kg, main effect (n = 32)						
500		78.40	29.83	6.20	9.74	27.02
1,500		78.45	29.86	6.17	9.80	26.91
CLM[5]		± 0.66	± 0.21	± 0.07	± 0.06	± 0.15
Microbiota modulating feed additives, main effect (n = 16)						
None		77.97	29.62	6.18	9.81	27.10
Only CB[6]		78.54	29.91	6.15	9.76	26.88
Only BMD[7]		78.39	30.01	6.22	9.72	26.93
Both CB and BMD		78.81	29.83	6.18	9.78	26.96
CLM		± 0.95	± 0.31	± 0.09	± 0.08	± 0.22
Phytase, FTU/kg x	Microbiota modulating feed additives					
500	None	78.08	29.63	6.19	9.74	27.03
500	Only CB	78.22	29.91	6.17	9.74	27.01
500	Only BMD	78.58	29.83	6.31	9.72	27.08
500	Both CB and BMD	78.73	29.97	6.12	9.75	26.97
1,500	None	77.85	29.62	6.17	9.89	27.16
1,500	Only CB	78.86	29.91	6.13	9.78	26.76
1,500	Only BMD	78.21	30.20	6.12	9.73	26.78
1,500	Both CB and BMD	78.90	29.70	6.24	9.80	26.96
CLM		± 1.35	± 0.44	± 0.14	± 0.12	± 0.32
				P-value		
Phytase		0.870	0.872	0.481	0.138	0.318
Microbiota modulating feed additives		0.665	0.326	0.799	0.451	0.542
Phytase x Microbiota modulating feed additives		0.884	0.540	0.134	0.676	0.458

[1] Carcass yields were calculated relative to 43 d live weight, while fillet, tender, wing, and leg yields were calculated relative to chilled carcass weight.

[2] Interaction values are least square means of 8 replicate pens, with each pen having 10 broilers processed on d 43.

[3] Fillet = combined weight of right and left *Pectoralis major*; Tender = combined weight of right and left *Pectoralis minor*; Wing = the wing tip, wing portion, and drumette; Leg = the drumstick and thigh.

[4] OptiPhos Plus (Huvepharma Inc., Peachtree City, GA) provided 500 FTU/kg or 1,500 FTU/kg of phytase activity per kg of diet.

[5] CLM = 95% confidence limit for the mean.

[6] ButiPEARL (Kemin Industries Inc., Des Moines, IA) included at 0 or 0.5 g supplement/kg of diet.

[7] Bacitracin methylene disalicylate 50 Type A Medicated Article (Zoetis, Parsippany, NJ) included at 0 or 55 mg per kg of diet.

Table 3.8. Independent orthogonal and orthogonal polynomial contrasts[1] for apparent ileal nutrient digestibility between YPM x Ross 708 male broilers provided a negative control diet (NC; 0 FTU/kg) and a NC with phytase supplementation (500 or 1,500 FTU/kg) at 28 d of age (grower phase)[2].

	Nutrient digestibility, %			AIDE[3], kcal/kg
Item	CP	Fat	Phosphorus	
NC vs. NC + phytase[4]				
NC	75.31	81.08	40.75	3,189
NC + 500 FTU/kg	76.67	85.37	56.24	3,289
NC + 1,500 FTU/kg	75.14	83.45	66.64	3,216
CLM[5]	± 1.35	± 2.81	± 2.67	± 61
	P-value			
NC vs. NC + 500 FTU/kg	0.118	0.047	<0.001	0.016
NC vs. NC + 1,500 FTU/kg	0.839	0.327	<0.001	0.515
Linear	0.590	0.521	<0.001	0.850
Quadratic	0.064	0.062	<0.001	0.011

[1] Orthogonal polynomial contrasts were performed for the unequally spaced phytase concentrations (0, 500, and 1,500 FTU/kg) without MMFA.

[2] Values are least square means of 8 replicate pens, with each pen having ileal digesta collected from 5 broilers on d 28; statistical significance was considered at $P \leq 0.05$.

[3] AIDE = apparent ileal digestible energy on a DM basis.

[4] OptiPhos Plus (Huvepharma Inc., Peachtree City, GA) provided 500 FTU/kg or 1,500 FTU/kg of phytase activity per kg of diet.

[5] CLM = 95% confidence limit for the mean.

Table 3.9. Apparent ileal nutrient digestibility of YPM x Ross 708 male broilers provided a negative control diet varying in phytase, calcium butyrate (CB), and bacitracin methylene disalicylate (BMD) inclusion at 28 d of age (grower phase)[1].

Item		Nutrient digestibility, %			AIDE[2], kcal/kg
		CP	Fat	Phosphorus	
Phytase[3], FTU/kg, main effect (n = 32)					
500		77.83	84.48	54.02	3,258
1,500		77.67	84.74	68.53	3,276
CLM[4]		± 0.64	± 1.22	± 1.25	± 28
Microbiota modulating feed additives, main effect (n = 16)					
None		75.90^{c}	84.41	61.44	3,253
Only CB[5]		77.19^{bc}	83.64	60.30	3,237
Only BMD[6]		78.64^{ab}	85.79	61.62	3,306
Both CB and BMD		79.26^{a}	84.60	61.74	3,272
CLM		± 0.90	± 1.79	± 1.87	± 40
Phytase, FTU/kg x	Microbiota modulating feed additives				
500	None	76.67	85.37	56.24^{b}	3,289^{ab}
500	Only CB	76.54	82.53	51.55^{b}	3,216^{b}
500	Only BMD	79.09	85.20	55.45^{b}	3,261^{ab}
500	Both CB and BMD	79.03	84.80	52.83^{b}	3,265^{ab}
1,500	None	75.14	83.45	66.64^{a}	3,216^{b}
1,500	Only CB	77.84	84.75	69.06^{a}	3,258^{ab}
1,500	Only BMD	78.19	86.38	67.79^{a}	3,351^{a}
1,500	Both CB and BMD	79.50	84.40	70.65^{a}	3,280^{ab}
CLM		± 1.32	± 2.53	± 2.74	± 58
		P-value			
Phytase		0.688	0.681	<0.001	0.352
Microbiota modulating feed additives		<0.001	0.353	0.634	0.086
Phytase x Microbiota modulating feed additives		0.103	0.381	0.007	0.038

[a-c] Means within a column with different superscripts differ significantly ($P \leq 0.05$).

[1] Interaction values are least square means of 8 replicate pens, with each pen having ileal digesta collected from 5 broilers on d 28.

[2] AIDE = apparent ileal digestible energy on a DM basis.

[3] OptiPhos Plus (Huvepharma Inc., Peachtree City, GA) provided 500 FTU/kg or 1,500 FTU/kg of phytase activity per kg of diet.

[4] CLM = 95% confidence limit for the mean.

[5] ButiPEARL (Kemin Industries Inc., Des Moines, IA) included at 0 or 0.5 g supplement/kg of diet.

[6] Bacitracin methylene disalicylate 50 Type A Medicated Article (Zoetis, Parsippany, NJ) included at 0 or 55 mg per kg of diet.

Table 3.10. Independent orthogonal and orthogonal polynomial contrasts[1] for apparent ileal nutrient digestibility between YPM x Ross 708 male broilers provided a negative control diet (NC; 0 FTU/kg) and a NC with phytase supplementation (500 or 1,500 FTU/kg) at 42 d of age (finisher phase)[2].

Item	Nutrient digestibility, %			AIDE[3], kcal/kg
	CP	Fat	Phosphorus	
NC vs. NC + phytase[4]				
NC	74.69	88.67	32.14	3,322
NC + 500 FTU/kg	76.70	90.21	55.11	3,354
NC + 1,500 FTU/kg	78.94	90.49	68.55	3,487
CLM[5]	± 1.98	± 1.56	± 3.55	± 60
	P-value			
NC vs. NC + 500 FTU/kg	0.135	0.191	<0.001	0.437
NC vs. NC + 1,500 FTU/kg	0.003	0.089	<0.001	<0.001
Linear	0.003	0.115	<0.001	<0.001
Quadratic	0.633	0.388	<0.001	0.515

[1] Orthogonal polynomial contrasts were performed for the unequally spaced phytase concentrations (0, 500, and 1,500 FTU/kg) without MMFA.

[2] Values are least square means of 8 replicate pens, with each pen having ileal digesta collected from 5 broilers on d 42; statistical significance was considered at $P \leq 0.05$.

[3] AIDE = apparent ileal digestible energy on a DM basis.

[4] OptiPhos Plus (Huvepharma Inc., Peachtree City, GA) provided 500 FTU/kg or 1,500 FTU/kg of phytase activity per kg of diet.

[5] CLM = 95% confidence limit for the mean.

Table 3.11. Apparent ileal nutrient digestibility of YPM x Ross 708 male broilers provided a negative control diet varying in phytase, calcium butyrate (CB), and bacitracin methylene disalicylate (BMD) inclusion at 42 d of age (finisher phase)[1].

		Nutrient digestibility, %			AIDE[2], kcal/kg
Item		CP	Fat	Phosphorus	
Phytase[3], FTU/kg, main effect (n = 32)					
500		76.64	89.84^{b}	54.89	3,376^{b}
1,500		77.79	90.95^{a}	69.71	3,479^{a}
CLM[4]		± 0.97	± 0.79	± 2.01	± 30
Microbiota modulating feed additives, main effect (n = 16)					
None		77.82	90.35	61.83	3,420
Only CB[5]		77.60	89.97	60.77	3,449
Only BMD[6]		77.19	90.37	64.40	3,404
Both CB and BMD		76.25	90.89	62.20	3,436
CLM		± 1.34	± 1.17	± 2.91	± 44
Phytase, FTU/kg x	Microbiota modulating feed additives				
500	None	76.70	90.21	55.11^{bc}	3,354
500	Only CB	76.79	89.22	50.37^{c}	3,397
500	Only BMD	76.89	90.17	59.53^{b}	3,354
500	Both CB and BMD	76.18	89.78	54.55^{bc}	3,399
1,500	None	78.94	90.49	68.55^{a}	3,487
1,500	Only CB	78.41	90.72	71.16^{a}	3,502
1,500	Only BMD	77.50	90.58	69.28^{a}	3,453
1,500	Both CB and BMD	76.31	92.00	69.85^{a}	3,473
CLM		± 1.96	± 1.72	± 4.57	± 66
		P-value			
Phytase		0.091	0.043	<0.001	<0.001
Microbiota modulating feed additives		0.354	0.617	0.290	0.475
Phytase x Microbiota modulating feed additives		0.672	0.591	0.046	0.785

[a-c]Means within a column with different superscripts differ significantly ($P \le 0.05$).

[1] Interaction values are least square means of 8 replicate pens, with each pen having ileal digesta collected from 5 broilers on d 42.

[2] AIDE = apparent ileal digestible energy on a DM basis.

[3] OptiPhos Plus (Huvepharma Inc., Peachtree City, GA) provided 500 FTU/kg or 1,500 FTU/kg of phytase activity per kg of diet.

[4] CLM = 95% confidence limit for the mean.

[5] ButiPEARL (Kemin Industries Inc., Des Moines, IA) included at 0 or 0.5 g supplement/kg of diet.

[6] Bacitracin methylene disalicylate 50 Type A Medicated Article (Zoetis, Parsippany, NJ) included at 0 or 55 mg per kg of diet.

IV. RESPONSE OF YPM X ROSS 708 MALE BROILERS TO DIETS CONTAINING VARYING INCLUSIONS OF PHYTASE, CALCIUM BUTYRATE, AND BACITRACIN METHYLENE DISALICYLATE DURING THE GROWER AND FINISHER PERIODS–PART 2: INTESTINAL HEALTH AND PHYSIOLOGY

ABSTRACT

This study evaluated the effects of calcium butyrate (**CB**) and bacitracin methylene disalicylate 50 (**BMD**) combined with different phytase concentrations on broiler intestinal health and physiology. Day-old YPM x Ross 708 male broilers (2,880) were distributed in 72 floor pens and assigned to 1 of 9 treatments (8 replicates/treatment). This experiment was a factorial arrangement including 2 phytase concentrations (500 or 1,500 FTU/kg) and 4 microbiota modulating feed additive levels (**MMFA**; (1) none, (2) only CB (0.5 g/kg of diet), (3) only BMD (55 mg/kg of diet), or (4) both CB and BMD). Additionally, a negative control without phytase and MMFA was included. Intestinal permeability was assessed on d 27. Jejunum wall and cecal content samples were collected on d 28 and 42 to assess jejunum villus height (**VH**), crypt depth, tight-junction and mucin gene expression, cecal microbiome diversity, and predicted bacterial metabolic pathways. Phytase and MMFA did not influence intestinal permeability ($P > 0.05$). Combining both CB and BMD with 1,500 FTU/kg of phytase compared to 500 FTU/kg lowered d 28 VH ($P \leq 0.05$). Jejunal expression of CL-1, CL-4, CL-5, and ZO-2 on d 28 as well as CL-2 on d 42 changed between MMFA when combined with 1,500 FTU/kg of phytase but not 500 FTU/kg ($P \leq 0.05$). Day 42 Pielou's evenness increased when 1,500 FTU/kg of phytase was combined with both CB and

BMD compared to no MMFA ($P \leq 0.05$). The cecal microbial beta diversity was not influenced by phytase, MMFA, or their interaction ($P > 0.05$). Overall, broiler intestinal health and physiology were influenced by CB and BMD depending on phytase concentration, demonstrating the complex interactions between these feed additives.

INTRODUCTION

The conversion of feed to muscle is the essence of broiler production. Until recently, antibiotic growth promotor (**AGP**) inclusion was an important strategy to improve production efficiency, weight gain, and feed conversion ratio (**FCR**) (Maria Cardinal et al., 2019). Bacitracin methylene disalicylate (**BMD**), a polypeptide antibiotic, has been traditionally used as an AGP to prevent diseases caused by gram-positive bacteria, such as *Clostridium perfringens* (Stutz et al., 1983). Its antimicrobial properties can help reduce pathogenic bacterial load in the intestine and modulate intestinal microbiota in a potentially positive way (Casewell et al., 2003; Crisol-Martínez et al., 2017). Due to concerns about antibacterial resistance and consumer pressure, AGP are no longer used or have limited use in most broiler-producing companies (Huyghebaert et al., 2011). However, BMD can still be used in the USA for prevention and control of necrotic enteritis.

One class of feed additives utilized to compensate for the loss of AGP are short chain fatty acids, among them butyric acid (Ayalew et al., 2022). Butyric acid or butyrate based feed additives can alter intestinal morphology (Deng et al., 2023), possess anti-inflammatory properties (Zou et al., 2019), and improve intestinal integrity (Mátis et al., 2022). They can also impact the composition and activity of the intestinal microbiota

(Ficagna et al., 2022; Wan et al., 2022), such as reducing *Salmonella* colonization and shedding (Fernández-Rubio et al., 2009; Onrust et al., 2020).

Phytase, a commonly used feed additive, is an enzyme that increases phosphorus availability by liberating phosphate from phytate and reducing its anti-nutritional effects of phytate in plant-based ingredients (Selle et al., 2023). Phytase can also indirectly affect intestinal health by increasing *Lactobacillus* colonization in the jejunum and ileum (Ptak et al., 2015), altering concentrations of short chain fatty acids and villus height (Moita et al., 2021), and having an effect on the systemic immune system (Liu et al., 2008).

To optimize broiler growth, it is important to understand how phytase interacts with calcium butyrate (**CB**) and BMD in relation to enteric health and physiology measurements. This experiment investigated the individual and interactive effects of these feed additives on intestinal permeability, jejunum histomorphometry and tight-junction gene expression, as well as the diversity and metagenomic analysis of the cecal microbiome in YPM x Ross 708 male broilers.

MATERIALS AND METHODS

Animal Care

This experiment was conducted at the Auburn University Charles C. Miller Jr. Poultry Research and Education Center. All procedures involving live birds were approved by the Auburn University Institutional Animal Care and Use Committee (PRN 2022-4061).

Bird Management

A total of 2,880 male d-old broiler chicks (YPM x Ross 708; Aviagen North America, Huntsville, AL) was obtained from a commercial hatchery. All chicks received a coccidiosis vaccine (Coccivac-B52, Merck Animal Health, Madison, NJ) containing live oocysts of *Eimeria* spp. (*E. acervulina*, *E. maxima*, *E. maxima MFP*, *E. mivati*, and *E. tenella*) at the hatchery. Upon arrival, chicks were weighed and distributed randomly among 72 floor pens (40 birds/pen, 0.07 m^2/bird from d 1 to 27; 33 birds/pen, 0.08 m^2/bird from d 28 to 42) in an environmentally controlled house. All floor pens contained used litter from 1 previous flock that was not subjected to a disease challenge. Each pen was top-dressed with new pine shavings. Feed and water were provided for *ad libitum* consumption.

Feed Formulation, Manufacture, and Experimental Design

Broilers were assigned to 1 of 9 treatments with 8 replicate pens per treatment. This experiment was a 2 x 4 + 1 factorial arrangement including 2 concentrations of phytase and 4 groups of microbiota modulating feed additives (**MMFA**). Phytase was included at either 500 or 1,500 FTU/kg (Optiphos Plus, Huvepharma Inc., Peachtree City, GA). Microbiota modulating feed additives included the individual or combined supplementation of a CB product (ButiPEARL, Kemin Industries Inc., Des Moines, IA) and BMD 50 (Zoetis, Parsippany, NJ). Therefore, the 4 groups of MMFA included no additive, CB (0.5 g supplement/kg of diet), BMD (55 mg/kg of diet), or both CB and BMD. The negative control (**NC**) was devoid of feed additives. The 8 treatments containing phytase, MMFA, or both were manufactured using the NC diet composition. The phytase product had an enzyme activity of 10,000 FTU/g, as indicated on the product packaging. According to the manufacturer, the encapsulated butyrate product contained 50% CB (0.5 g supplement/kg of diet = 0.25 g CB/kg of diet). As for BMD, 454 g of product was equivalent to 50 g of bacitracin. Ingredient and nutrient composition of experimental diets are presented in a companion manuscript with live performance measurements (see Chapter III; Gulizia et al., 2024). Broilers were fed a 3-phase feeding program with starter feed from d 1 to 14, grower feed from d 15 to 28, and finisher feed from d 29 to 42.

Intestinal Permeability

A modified intestinal permeability protocol was followed in accordance with Baxter et al. (2017). On d 27, fluorescein isothiocyanate dextran (**FITC-d**; Sigma Aldrich Co., St. Louis, MO) was administered in a 1 mL oral gavage (8.3 mg/kg of BW) to 2 randomly selected birds per replicate. The solution was applied directly into the crop using a 1 mL-insulin syringe with a buttoned cannula attached. Birds did not undergo a fasting period before receiving the solution to avoid compromising intestinal integrity or confounding other intestinal samples (Liu et al., 2021). After 2 h, approximately 1 mL of blood was taken from the wing vein using a syringe and hypodermic needle and collected in heparin tubes (Sarstedt, Nümbrecht, Germany). Blood samples were directly placed into a cooler after collection. Samples were centrifuged (VWR International, Radnor, PA) at 1,000 x g for 15 min to isolate the plasma. Afterwards, plasma samples were analyzed for fluorescence using a SpectraMax iD3 microplate reader (Molecular Devices, San Jose, CA). To determine FITC-d concentrations, a standard calibration curve was produced using sterile PBS (VWR, Solon, OH) and known concentrations of FITC-d at a 2-fold dilution series (0.00000, 0.00063, 0.00125, 0.00250, 0.00500, 0.01000, 0.02000, and 0.04000 mg/mL) in triplicates. For both the standard curve and sample plates, the plate read configuration had an excitation wavelength of 485 nm with an emission wavelength of 528 nm.

Sampling

Five birds per replicate were randomly selected and euthanized using carbon dioxide followed by cervical dislocation on d 28 and 42. The gastrointestinal tract was removed, and the jejunum and ceca were isolated. Approximately 10 cm upstream of Meckel’s diverticulum, a section of approximately 2 cm was flushed with sterile PBS (VWR, Solon, OH) and then placed into 10% buffered formalin (VWR, Radnor, PA). From these same 5 birds, 1 g of adjacent jejunal wall was collected from 2 birds per replicate and stored for 24 h in RNAlater (Qiagen, Hilden, Germany) at 4°C. After that, RNAlater was discarded, and the sample was stored at −80°C for relative gene expression analysis. Cecal content was also collected from 2 of the 5 birds per replicate, which were randomly selected from the original 5 euthanized birds. This cecal content was collected using plastic spoons to minimize contamination with chicken DNA, immediately placed on ice, and then stored at −80°C for microbiome analysis.

Jejunum Histomorphometry

Day 28 and 42 jejunum samples were processed by the Alabama State Diagnostic Laboratory at Auburn University (Auburn, AL). Samples were embedded in paraffin, sectioned at 5 µm, mounted on glass slides, stained with hematoxylin and eosin by routine methods, and were evaluated using light microscopy. Photomicrographs were taken at 40x magnification. The height of 10 villi and the depth of 10 adjacent crypts per pen (5 birds/pen) were measured using Olympus cellSens Standard image software (version 1.12, 2014). Individual villi were randomly selected, apparently complete, and full-sized without bending or mechanical damage. Villus height (**VH**) and crypt depth (**CD**) were recorded and VH to CD ratios (**VH:CD**) were calculated. All jejunum histomorphometry measurements were averaged for each pen with the pen being the statistical unit.

Jejunum Gene Expression

Ribonucleic acid was extracted from 15 mg of jejunal wall with the Qiagen RNeasy kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions with DNA digestion on the column during RNA extraction with the Qiagen RNase-Free DNase Set (Qiagen, Hilden, Germany). While 2 birds per replicate were sampled, only 1 randomly selected sample per replicate plus 2 randomly selected samples (total of 10 samples/treatment) were used for analysis. Total RNA was transcribed into cDNA using the Lunascript kit (New England Biolabs, Ipswich, MA). The cDNA was diluted 1:4 in RNAse-free water. Two housekeeping genes (GAPDH and HMBS), 6 tight junction genes including claudins (CL-1, CL-2, CL-4, and CL-5) and zonula occludens (ZO-1 and ZO-2), and 1 mucus production gene (MUC-2) were amplified in duplicates by qPCR using the Fast SYBR Green Master Mix (Qiagen, Hilden, Germany). Cycling parameters consisted of an initial 10 min at 95°C denaturation cycle, 40 cycles of denaturation for 30 s at 95°C, annealing for 1 min at 60°C, and extension at 72°C for 30 s. Specificity of PCR products was confirmed by a melting curve analysis. To obtain each primer efficiency, a standard curve was calculated for each gene with a 2-fold dilution. Primers, their efficiencies, and references are listed in **Table 4.1**. Gene expression was calculated relative to the expression of 2 housekeeping genes (GAPDH and HMBS), with the average of the control birds used as a calibrator, following the method described by Vandesompele et al. (2002) and Hellemans et al. (2007).

Cecal Microbiome

Cecal content was sampled from 2 birds per replicate. However, only 1 randomly selected sample per replicate (8 samples/treatment) was used for analysis. Deoxyribonucleic acid was extracted from 200 µL cecal content using the QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. Between 400 and 450 base pairs (**bp**) of the bacterial 16S rRNA gene were amplified as described by Hamilton et al. (2019) using the Taq PCR Master Mix Kit (Qiagen, Hilden, Germany). Primers 515F with linker CS1 and 926R with linker CS2 were used. Polymerase chain reaction products were verified by agarose gel electrophoresis and submitted to the University of Illinois at Chicago DNA Services Facility (Chicago, IL) for next-generation amplicon sequencing on the Illumina MiSeq. The raw reads were deposited into the NCBI Sequence Read Archive database (BioProject ID[1]: PRJNA1206084).

For pre-processing of the raw reads, taxonomic classification, and analysis of diversity core metrics, a standard QIIME2 pipeline was utilized (Bolyen et al., 2019). The primers were trimmed from the reads, and sequences were denoised using a truncation length of 250 bp and a maximum expected error threshold of 4 for both forward and reverse reads using the DADA2 plugin. The taxonomic composition of the bacterial communities was assessed using the Naïve Bayes classifier trained on Silva Release 138 sourced from the SILVA ribosomal RNA gene database project (Quast et al., 2013). Sequences were filtered at a threshold of 20,000 reads for generating core diversity metrics. Cecal microbiome alpha diversity was assessed for community evenness using Pielou's evenness index (Pielou, 1966), phylogeny-based richness using Faith's

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[1] The accession includes additional samples that were excluded from the cecal microbiome analyses, as only 1 bird per pen was analyzed.

phylogenetic diversity index (**FPD**; Faith, 1992), and non-phylogenetic richness termed as observed features (**OF**) in the QIIME2 program (Bolyen et al., 2019). Unweighted UniFrac distances were used to evaluate cecal microbiome beta diversity (Lozupone and Knight, 2005).

The bacterial metagenome prediction was performed using PICRUSt2 program (Douglas et al., 2020). Predicted metagenome consisted of gene names derived from Kyoto Encyclopedia of Genes and Genomes (**KEGG**) Orthology Database (Kanehisa et al., 2017). The subsequent analyses were carried out using RStudio version 2024.04.1 Build 748 (R Core Team, 2023; Posit team, 2024). The differential expression of predicted genes was computed using DESeq2 package (Love et al., 2014). Differentially expressed genes (**DEGs**) were filtered using statistical significance thresholds $P < 0.05$ and log2 fold change ≥ 1 for respective group comparisons of phytase and MMFA. Genes with a log2 fold change > 1 were considered enriched, while a log2 fold change < 1 were depleted. For pathway annotation, ClusterProfiler 4.0 (Wu et al., 2021) package was utilized. Briefly, a ranked list of DEGs was prepared based on log2 fold change values and fed into the enrichKEGG function. Top bacterial metabolic pathways were selected from relevant subcategories based on the gene ratio, and dot plots were generated using the ggplot2 package (Wickham, 2016) in RStudio version 2024.04.1 Build 748 (R Core Team, 2023; Posit team, 2024).

Statistical Analyses

This experiment was a completely randomized design with data analyzed as a 2 x 4 factorial to evaluate the main effects of phytase and MMFA and their interactions. There were 2 concentrations of phytase (500 or 1,500 FTU/kg) and 4 groups of MMFA (none, only CB, only BMD, or both CB and BMD). Excluded from this factorial analysis was the NC treatment. Instead, independent orthogonal contrasts were performed for comparison between phytase supplemented diets with no MMFA (NC + 500 FTU/kg and NC + 1,500 FTU/kg) and the NC (0 FTU/kg). In addition, orthogonal polynomial contrasts were performed for the unequally spaced phytase concentrations (0, 500, and 1,500 FTU/kg). The ORPOL function in PROC IML was used for determination of CONTRAST coefficients for the unequally spaced treatments. Independent orthogonal and orthogonal polynomial contrasts were conducted for all data except for cecal microbiome beta diversity and metagenomic analyses.

Each treatment was represented by 8 replicate pens. Pen was considered as the experimental unit for intestinal permeability, jejunum histomorphometry, cecal microbiome diversity, and metagenomic analysis, whereas bird was the experimental unit for tight-junction gene expression. Relative gene expression values were log2 transformed before statistical analysis. Using Tukey's method, outliers were determined when values were 1.5 times the interquartile range below and above the first and third quartile, respectively (Tukey, 1977). Plasma fluorescence, histomorphometry, log2 transformed relative gene expression, and cecal microbiome alpha diversity were analyzed using the GLIMMIX procedure of the SAS software version 9.4 (SAS Institute Inc., Cary, NC). Least square means were compared using the Tukey-Kramer option with statistical significance considered at $P \leq 0.05$. For all means, 95% confidence limits were determined. If the Tukey-Kramer test was unable to separate the means, then interactions will be described by determining the overlap of 95% confidence limits between treatments (Cumming and Fidler, 2009).

The cecal microbiome beta diversity indices were statistically compared using UniFrac distance matrix (Lozupone and Knight, 2005) between the NC (0 FTU/kg) and NC with phytase inclusion (500 and 1,500 FTU/kg) and with a factorial design approach excluding the NC. Similarly, cecal microbiome beta diversity was compared between sampling days using the interaction treatments (**Appendix 1**). To determine statistical significance in a multivariate analysis and generate dispersion plots, adonis function within the vegan package (Oksanen et al., 2015) was used for permutational analysis of variance in the statistical software RStudio version 2024.04.1 Build 748 (R Core Team, 2023; Posit team, 2024). Differential abundance of various genera was tested using Analysis of Compositions of Microbiomes with Bias Correction package version 2.6.0 (Lin and Peddada, 2020) (**Appendix 1**). The analysis included feature filtering, zero-inflation adjustment, and compositional bias correction. The output included fold changes of various taxa compared to NC group.

Analysis of differential expression of PICRUSt2-predicted genes involved a negative binomial distribution model in DESeq2 package to estimate variance-mean dependence in count data. ClusterProfiler utilizes hypergeometric tests to assess the over-representation of KEGG pathways among the DEGs and reports *P*-values following adjustment for multiple comparisons using the Benjamini-Hochberg method. A detailed account of statistics for DEGs (**Appendix 2**) and pathways (**Appendix 3**) were reported.

To determine the relationship between d 28 and 42 live performance measurements reported in Gulizia et al. (2024) and intestinal health and physiology measurements, Pearson correlation coefficients (**Appendix 1**) were generated using the CORR procedure of the SAS software version 9.4 (SAS Institute Inc., Cary, NC). The analysis was performed across phytase and MMFA main effects and with the pen as the statistical unit. Strength of the correlation was determined based on the description by Pang et al. (2024): weak (|r| = 0.20-0.39), moderate (|r| = 0.40-0.59), strong (|r| = 0.60-0.79), and very strong (|r| = 0.80-0.99). The performance measurements considered for analysis were BW, FCR, CP digestibility, and apparent ileal digestible energy (**AIDE**), whereas the intestinal health and physiology measurements were VH, VH:CD ratio, Pielou's evenness index, and OF.

RESULTS

Intestinal Permeability

Independent orthogonal and orthogonal polynomial contrasts between the NC (0 FTU/kg) and NC with phytase inclusion (500 and 1,500 FTU/kg) on intestinal permeability were not different ($P > 0.05$) (**Table 4.2**). Broiler intestinal permeability on d 27 was not influenced by phytase or MMFA ($P > 0.05$) (**Table 4.3**).

Jejunum Histomorphometry

Independent orthogonal and orthogonal polynomial contrasts between the NC (0 FTU/kg) and NC with phytase inclusion (500 and 1,500 FTU/kg) on d 28 histomorphometry were not different ($P > 0.05$) (**Table 4.2**). However, there were differences between the NC and NC with phytase inclusion on d 42 histomorphometry as indicated by the independent orthogonal and orthogonal polynomial contrasts (**Table 4.2**). Broilers consuming the NC devoid of phytase had an increased d 42 CD and lowered VH:CD ratio compared to both phytase supplemented groups ($P \leq 0.05$). A quadratic response to phytase supplementation was observed for d 42 CD ($P \leq 0.05$).

Day 28 and 42 jejunum VH, CD, and VH:CD ratios are shown in **Table 4.3**. Interactions between phytase and MMFA were observed for d 28 VH and VH:CD ratio ($P \leq 0.05$). Within each phytase group, d 28 VH was similar between all levels of MMFA. However, d 28 VH increased when both CB and BMD were combined with 500 FTU/kg of phytase compared to 1,500 FTU/kg ($P \leq 0.05$). At a phytase concentration of 1,500 FTU/kg, d 28 VH:CD ratio across treatments showed minimal variation. However, with 500 FTU/kg of phytase, including both CB and BMD in the diet increased d 28 VH:CD ratio compared to only CB ($P \leq 0.05$). Phytase and MMFA did not influence d 28 CD ($P > 0.05$). Irrespective of MMFA, d 42 jejunum histomorphometry was not different between phytase concentrations ($P > 0.05$). Only d 42 VH was influenced by MMFA. Including only CB in the diet increased VH compared to including either only BMD or both CB and BMD ($P \leq 0.05$).

Jejunum Gene Expression

Only d 28 CL-1 and CL-2 expressions were different between the NC (0 FTU/kg) and NC with phytase inclusion (500 and 1,500 FTU/kg) as indicated by the independent orthogonal and orthogonal polynomial contrasts (**Table 4.4**). Claudin-1 was downregulated more when broilers consumed NC + 1,500 FTU/kg compared to the NC with no phytase ($P \leq 0.05$). A linear response to increasing phytase concentrations was observed for CL-1 expression ($P \leq 0.05$). More pronounced downregulation of CL-1 expression occurred as phytase concentrations increased. Additionally, downregulation of CL-2 occurred when broilers consumed the NC + 500 FTU/kg compared to NC ($P \leq 0.05$).

Dietary inclusion of phytase and MMFA influenced d 28 tight junction gene expression (**Table 4.5**). Compared to no inclusion of MMFA, CL-2 expression was upregulated with dietary inclusion of only BMD ($P \leq 0.05$). Phytase and MMFA interactions were observed for CL-1, CL-4, CL-5, and ZO-2 expression ($P \leq 0.05$). Within the 500 FTU/kg group, expressions of CL-1, CL-4, CL-5, and ZO-2 were not influenced by CB, BMD, and their combination. Therefore, MMFA responded differently with a dietary inclusion of 1,500 FTU/kg of phytase. Claudin-1 expression was highest with the inclusion of only CB. Additionally, CL-1 expression increased with only BMD compared to no MMFA ($P \leq 0.05$). Supplementation of only CB increased CL-4 and ZO-2 expression compared to the other MMFA groups ($P \leq 0.05$). Claudin-5 expression was higher when broilers consumed a diet with only CB compared to no MMFA ($P \leq 0.05$).

There were multiple differences between the NC (0 FTU/kg) and NC with phytase inclusion (500 and 1,500 FTU/kg) on d 42 tight junction and mucin gene expressions as indicated by the independent orthogonal and orthogonal polynomial contrasts (**Table 4.6**). Day 42 CL-2 expression was more downregulated when broilers consumed the NC diet with 1,500 FTU/kg compared to 0 FTU/kg ($P \leq 0.05$). A linear response to increasing phytase concentrations was observed for CL-2 expression ($P \leq 0.05$). As phytase concentrations increased, a more pronounced downregulation of CL-2 expression occurred. Compared to a diet devoid of phytase, ZO-2 expression was downregulated with dietary inclusion of either 500 or 1,500 FTU/kg ($P \leq 0.05$; Linear: $P \leq 0.05$). Lastly, dietary inclusion of 1,500 FTU/kg of phytase downregulated MUC-2 compared to NC ($P \leq 0.05$). Day 42 expression of MUC-2 linearly decreased with higher concentrations of phytase ($P \leq 0.05$).

Day 42 MUC-2 expression was upregulated with 500 FTU/kg compared to 1,500 FTU/kg (**Table 4.7**) ($P \leq 0.05$). Irrespective of phytase, inclusion of both CB and BMD upregulated CL-1 while including single or combinations of MMFA reduced the degree of downregulation for ZO-2 ($P \leq 0.05$). Expression of MUC-2 was upregulated with dietary supplementation of only CB compared to no MMFA ($P \leq 0.05$). A phytase and MMFA interaction was only observed for d 42 CL-2 expression ($P \leq 0.05$). Expression of CL-2 was not influenced by MMFA in the 500 FTU/kg group. However, within the 1,500 FTU/kg group, the degree of downregulation was reduced with only CB compared to no MMFA ($P \leq 0.05$).

Cecal Microbiome

Relative abundance of the 10 most abundant genera in the cecal microbiome on d 28 and 42 are shown in **Figure 4.1** (**a**) and (**b**), respectively. Excluding the others, *Alistipes*, *Faecalibacterium*, and *Ruminococcaceae UCG-014* were the most common genera found in the cecal microbiome on d 28, whereas *Alistipes*, *Bacteroides*, and *Faecalibacterium* were the most abundant on d 42. For both d 28 and 42, there were no apparent differences between the treatments. Additional details on the log fold change in bacterial genera are provided in **Appendix 1** (**Figure S4.1**).

Independent orthogonal and orthogonal polynomial contrasts between the NC and NC with phytase inclusion were not different for d 28 and 42 cecal microbiome alpha diversity characteristics ($P > 0.05$) (**Table 4.8**), except for d 28 Pielou's evenness, which quadratically responded to phytase concentrations ($P \leq 0.05$).

Day 28 and 42 cecal microbiome alpha diversity characteristics are shown in **Table 4.9**. A higher d 28 OF was observed with dietary inclusion of 1,500 FTU/kg of phytase compared to 500 FTU/kg ($P \leq 0.05$). An interaction between phytase and MMFA was only observed for Pielou's evenness on d 28 ($P \leq 0.05$). Within the 500 FTU/kg group, evenness was increased with inclusion of only BMD compared to only CB and no MMFA ($P \leq 0.05$). In contrast, evenness was similar between all MMFA within the 1,500 FTU/kg group. Phytase, MMFA, and their combination did not influence d 42 FPD ($P > 0.05$). However, interactions were observed for d 42 Pielou's evenness and OF ($P \leq 0.05$). Microbiota modulating feed additives did not influence evenness within the 500 FTU/kg group. However, within the 1,500 FTU/kg group, d 42 evenness increased when supplementing both CB and BMD compared to no MMFA ($P \leq 0.05$). Although an interaction was observed for d 42 OF, the Tukey-Kramer multiple comparison test did not separate the means, and the 95% confidence intervals overlapped across all treatments.

Day 28 ($P = 0.470$) and 42 ($P = 0.148$) cecal microbiome beta diversity was not different between the NC (0 FTU/kg) and NC with 500 or 1,500 FTU/kg of phytase (data not shown). Phytase ($P = 0.371$), MMFA ($P = 0.196$), and their interaction ($P = 0.175$; **Appendix 1, Figure S4.2**) did not influence d 28 cecal microbiome beta diversity (main effect data not shown). Similarly for d 42 cecal microbiome beta diversity, phytase ($P = 0.695$), MMFA ($P = 0.642$), and their interaction ($P = 0.070$; **Appendix 1, Figure S4.3**) were not different (main effect data not shown).

Number of up- and down-regulated DEGs based on predicted metagenome of the cecal microbiota compared between the NC (0 FTU/kg) and NC with phytase inclusion (500 and 1,500 FTU/kg) is shown in **Table 4.10**, while the influence of phytase and MMFA on DEGs is presented in **Table 4.11**. Supplementing phytase at 500 FTU/kg (d 28: 103 DEGs compared to the NC; d 42: 1,340 DEGs compared to the NC) and 1,500

FTU/kg (d 28: 184 DEGs compared to the NC) had the most influence on the inferred metabolic activity of the microbiota. However, the effect was inconsistent, with the lower dose of phytase having a much higher impact on d 42 and the higher dose showing a moderate impact on d 28. Furthermore, there were 2 common DEGs between d 28 and 42 of the 1,443 genes influenced by 500 FTU/kg of phytase. These DEGs were involved in small acid-soluble spore protein I (K06426) and two-component system, OmpR family, sensor histidine kinase ArlS (K18940). Between d 28 and 42, only 58 unique DEGs were observed from the dietary inclusion of CB, BMD, or both feed additives compared to no MMFA.

Selected predicted bacterial metabolic pathways between the NC + phytase groups and the NC without phytase on d 28 and 42 are shown in **Figures 2**, **3**, **4**, and **5**. No pathways primarily associated with phosphorus or calcium absorption or phytate degradation were regulated with 500 or 1,500 FTU/kg of phytase. However, depending on sampling day, dietary phytase either upregulated or downregulated pathways involved in carbohydrate metabolism, metabolism of cofactors and vitamins, energy metabolism, amino acid metabolism, and lipid metabolism. In the cecal microbiome on d 28, dietary phytase at 500 FTU/kg downregulated metabolic pathways related to pyruvate metabolism, methane metabolism, Gly, Ser, and Thr metabolism, and glycerophospholipid metabolism (**Figure 4.2**). Supplementing 1,500 FTU/kg of phytase upregulated pathways related to pyruvate metabolism, other carbon fixation pathways, lipoic acid metabolism, Gly, Ser, and Thr metabolism, and fatty acid degradation (**Figure 4.3**). Conversely, on d 28 using 1,500 FTU/kg, downregulation was observed in pathways associated with Val, Leu, and Ile degradation, pyruvate metabolism, other carbon fixation pathways, lipoic acid metabolism, and glycerophospholipid metabolism (**Figure 4.4**). As for the cecal microbiome on d 42, dietary phytase at 500 FTU/kg downregulated pyruvate metabolism, porphyrin metabolism, oxidative phosphorylation, Gly, Ser, and Thr metabolism, and glycerophospholipid metabolism (**Figure 4.5**). Among these pathways, pyruvate metabolism, Gly, Ser, and Thr metabolism, lipoic acid metabolism, and glycerophospholipid metabolism were observed across multiple NC + phytase and NC comparisons. Detailed cecal microbiome metabolic pathway analyses comparing NC and NC + phytase inclusion are provided in **Appendix 3**.

Pearson correlation coefficients between d 28 and 42 live performance measurements and intestinal health and physiology measurements are presented in **Tables S4.1** and **S4.2** (**Appendix 1**). On d 28, most correlations occurred in the 500 FTU/kg group, including a weak positive correlation between VH and BW, moderate positive correlations between both VH:CD ratio and Pielou’s evenness with BW, and a moderate negative correlation between Pielou’s evenness and FCR ($P \leq 0.05$). In the 1,500 FTU/kg group, OF showed a moderate positive correlation with BW, while VH:CD ratio had a strong positive correlation with BW in the only BMD group ($P \leq 0.05$). Pielou’s evenness (only CB) and VH (both CB and BMD) had a moderate negative and positive correlation with FCR, respectively ($P \leq 0.05$). In the group without MMFA, Pielou’s evenness showed a strong negative correlation with AIDE ($P \leq 0.05$). On d 42, VH showed a moderate positive correlation with CP digestibility (500 FTU/kg and only BMD) and AIDE (1,500 FTU/kg), whereas a moderate negative correlation was observed with BW (500 FTU/kg) ($P \leq 0.05$). In the 1,500 FTU/kg and only BMD groups, VH:CD ratio had a weak and strong positive correlation with AIDE and CP digestibility, respectively ($P \leq 0.05$). Pielou's evenness showed a strong negative (only CB) and moderate positive (both CB and BMD) correlation with FCR and AIDE, respectively ($P \leq 0.05$). Observed features showed a moderate negative correlation with AIDE in the group without MMFA ($P \leq 0.05$).

DISCUSSION

Phytase, CB, and BMD are frequently used as feed additives to improve the performance of broilers. Analysis of bird performance and nutrient digestibility indicated that BMD had a more positive impact compared to CB and revealed that broiler growth response to CB and BMD can be influenced by phytase concentration (see Chapter III; Gulizia et al., 2024). As will be discussed later, intestinal health and physiology measurements responded more to phytase and CB than to BMD. Consequently, directly linking the present results to the live performance outcomes reported in Chapter III (Gulizia et al., 2024) is challenging. This is also supported by the Pearson correlation coefficients, which showed no consistent pattern of correlations between measurements on d 28 and 42. However, the 500 FTU/kg phytase group appeared more likely to exhibit correlations between these measurements. Thus, establishing a direct relationship between intestinal health and physiology measurements and live performance outcomes in response to these dietary interventions is complex. Nevertheless, the analyses of these data presented herein were undertaken to explore how phytase, CB, and BMD affect diversity and function of the intestinal microbiota, intestinal permeability, histomorphometry, and expression of tight junction genes.

Butyrate-based feed additives and BMD act mostly by modifying the intestinal microbiota (Bortoluzzi et al., 2017; Li et al., 2022), which then can change expression of tight junction genes, histomorphometry, and intestinal permeability. In addition, butyrate-based feed additives can have immunomodulatory effects (Zhou et al., 2014; Bortoluzzi et al., 2017). In contrast, phytase acts on the feed by degrading phytate, which releases phosphorus and reduces its anti-nutritional properties related to endogenous nitrogen losses (Cowieson et al., 2004; Cowieson et al., 2009). Reducing the intestinal flow of undigested protein and nitrogen can limit the substrate available for pathogenic bacteria (Qaisrani et al., 2015). Similarly, lowering endogenous nitrogen losses through phytase-mediated phytate degradation may also restrict the growth of pathogenic bacteria (Ptak et al., 2015; Moita et al., 2021). In this context, the effects of phytase, CB, and BMD on intestinal microbiota are of central importance. These effects have been studied before and yielded mostly similar results compared to the present study. Ultimately, the key results of this study are the interactions between phytase and the MMFA. In agreement with literature (Proctor and Phillips, 2019; Naghizadeh et al., 2022a), CB and BMD generally did not change alpha diversity of the cecal microbiota. However, interactions were observed with an alpha diversity response to the inclusion of MMFA in the 500 FTU/kg phytase group on d 28, while a response to MMFA occurred in the 1,500 FTU/kg group on d 42. The inclusion of phytase or MMFA did not significantly change the composition of the cecal microbiota, as assessed by beta diversity. Lack of a significant effect from MMFA, i.e. feed additives intended to modulate the intestinal microbiota, on beta diversity was unexpected, but not unprecedented (Adhikari et al., 2023; Melaku et al., 2024). Alpha and beta diversity responses of the cecal microbiota to specific dietary feed additives can depend on the inclusion of additional feed additives, potentially explaining conflicting results in the literature.

Regulation of bacterial metabolic pathways is the most important aspect of the microbiome, potentially affecting host-microbiome interactions (Pan and Yu, 2014). There are limited papers which assessed the effects of phytase on predicted metabolic pathways in the intestinal microbiota of chickens. Depending on the concentration, phytase had a more pronounced effect on DEGs and predicted metabolic functions in the cecal microbiome compared to the MMFA. Complete dephosphorylation of phytate can liberate inositol (Kriseldi et al., 2021), which can further influence the intestinal microbiota (Yan et al., 2024). In the present study, phytase upregulated Gly, Ser, and Thr metabolism, which is the opposite result of Siegert et al. (2021) who reported that 1,500 FTU/kg of phytase decreased Gly, Ser, and Thr metabolism in the ileum microbiota. The reasons for conflicting results are unclear and may be related to the dietary inclusion of MMFA or the intestinal section sampled. In addition, the analytical tools used for assessing bacterial metabolic pathways limited the evaluation of interactions, hindering a clear understanding of how metabolic functions are regulated when both phytase and MMFA are supplemented. Siegert et al. (2021) also showed interactions between phytase and calcium levels, making a direct comparison difficult.

Exogenous phytase also changed pyruvate metabolism and lipoic acid metabolism within the cecal microbiome. Pyruvate metabolism is associated with energy production pathways (Turnbaugh et al., 2008), while lipoic acid is a necessary cofactor for reactions in pyruvate metabolism (Reed, 1957). Glycine, Ser, and Thr are glucogenic amino acids that have interconnected pathways with pyruvate, contributing to both amino acid and energy metabolism (Woolfson, 1983; He et al., 2021). Additionally, pyruvate metabolism has been positively correlated with *Alistipes* (Zhang et al., 2019), the most common genus observed on d 28 and 42 in the present study. All of this indicates that altered pyruvate metabolism is central to the observed changes in cecal microbiota function. Furthermore, glycerophospholipid metabolism was the only pathway consistently downregulated with phytase inclusion. Metabolites of glycerophospholipid metabolism have important roles in lipid metabolism, cellular membrane integrity, and signaling (Denich et al., 2003; Zhang and Rock, 2008). Upstream intestinal development, nutrient digestibility, and subsequent flow of substrates to the lower gastrointestinal tract may influence downstream microbial dynamics (Apajalahti et al., 2012; Quinger et al., 2024). The minimal effects of phytase and MMFA on the cecal microbiota suggest limited treatment impacts on intestinal permeability, histomorphometry, and tight junction gene expression. Further investigation is needed to understand the relationship between cecal microbiome dynamics and upper small intestinal physiology.

Intestinal permeability was not altered, and this lack of response to the addition of phytase or MMFA has been previously observed (Zanu et al., 2020a; Naghizadeh et al., 2022b). Another method to assess intestinal health is through the morphological development of the gastrointestinal tract, where higher VH and lower CD indicate better intestinal health and improved nutrient absorption capacity (Ducatelle et al., 2018; Bindari and Gerber, 2022). Some available literature suggests that phytase (Moita et al., 2021) and butyrate-based feed additives (Ahsan et al., 2016) can enhance intestinal VH and VH:CD ratio. Previous research also indicates that bacitracin alone did not improve intestinal morphology (Letlole et al., 2021; Adhikari et al., 2023). These findings are only in partial agreement with the results of the present study. While CB increased d 42 jejunal VH compared to BMD, phytase affected jejunal histomorphometry only on d 42 in birds not supplemented with MMFA. As with the cecal microbiota, one possible cause of these partial discrepancies may be interactions between dietary feed additives, some of which were also observed in the present study. Combining 1,500 FTU/kg of phytase with both CB and BMD decreased d 28 VH compared to 500 FTU/kg. Similarly, higher phytase concentrations combined with sodium butyrate (1,500 vs. 750 FTU/kg; Layter et al., 2021) increased CD, while higher phytase concentrations combined with both salinomycin and zinc bacitracin (1,500 vs. 500 FTU/kg; Zanu et al., 2020b) reduced VH. Given the limited literature, there is possibly an extent to which higher phytase concentrations combined with butyrate or antibiotics can improve intestinal histomorphometry, though the specific mechanism remains unclear.

The final set of measurements evaluated were the expression of tight junction and mucin producing genes, which play critical roles in maintaining intestinal integrity and function by regulating the paracellular movement of ions (González-Mariscal et al., 2003; Awad et al., 2017). Claudins 1, 4, and 5 are barrier-forming tight junction proteins that can prevent paracellular movement of cations, pathogens, and feed contaminants in the gastrointestinal tract, while CL-2 has a pore-forming role that can facilitate the movement of cations across the epithelium (Günzel and Yu, 2013; Awad et al., 2017). Zonula occludens support the structure and function of claudins (González-Mariscal et al., 2003; Lee, 2015), with ZO-1 and ZO-2 having independent effects on claudins during tight junction formation (Umeda et al., 2006). Although not a tight junction gene, MUC-2 is crucial for mucin production, which serves as the first line of defense against pathogens in the gastrointestinal tract (Jiang et al., 2013; Duangnumsawang et al., 2021). Even more than the previously discussed factors, reports on the influence of phytase and MMFA on intestinal tight junction and mucin gene expression are conflicting. These effects vary depending on dosing level, other dietary factors (Zanu et al., 2020a; Luo et al., 2021), and disease state (Song et al., 2017; Shi et al., 2024). The complexity is evident in the present findings, where interactions were observed in the expression of CL-1, CL-4, CL-5, and ZO-2 on d 28 as well as CL-2 on d 42. Interestingly, less interactions were observed on d 42, suggesting that the effect of phytase and MMFA becomes more uniform in older birds with a more mature intestinal microbiota. While it remains unclear whether the up- or down-regulation of these genes benefits intestinal health, and no optimal level has been established, the regulation of tight junction gene expression appears to have supported intestinal integrity, as no differences were observed in intestinal permeability. Furthermore, expression of tight junction and mucin genes were altered but this does not always refer to protein translation (Greenbaum et al., 2003).

In the present study, more interaction effects on jejunal histomorphometry, jejunal gene expression, and cecal alpha diversity were observed on d 28 compared to d 42 (7 vs. 3 significant interactions). Possibly, the effects of phytase and MMFA were influenced by bird age and gastrointestinal tract physiology. Discrepancies between sampling days could be attributed to shifts in microbial populations (Lu et al., 2003), age-related changes in intestinal tight junction protein expression (von Buchholz et al., 2021; Abascal-Ponciano et al., 2022), and alterations in diet composition (Marmion et al., 2021). Although the jejunal microbiome was not assessed, a distinct shift in cecal microbiome beta diversity between sampling days (**Appendix 1, Figure S4.4**) suggests that microbial communities may have also changed over time. This shift over time is supported by the log fold change in genera (**Appendix 1, Figure S4.1**) as dietary treatments had less influence on d 42 as compared to d 28. As broilers age, their intestinal development (Ravindran and Abdollahi, 2021) and microbial populations (Lumpkins et al., 2010; Rama et al., 2023) tend to stabilize, which may explain the reduced sensitivity to dietary interventions observed on d 42 in this study. However, the specific timeframe for microbial stabilization in broilers remains ambiguous (Feye et al., 2020).

Dietary supplementation of phytase (500 or 1,500 FTU/kg), CB (0.5 g supplement/kg of diet), and BMD (55 ppm) influenced indicators of intestinal health and physiology of broilers. While intestinal permeability remained unaffected and changes in jejunal histomorphometry and cecal microbiome diversity were minimal, phytase, MMFA, and, at times, their interaction, significantly affected jejunal tight junction and mucin gene expression and predicted bacterial metabolic function. In conclusion, these findings highlight the complex interactions between feed additives, particularly the dependence of MMFA effects on phytase concentration. Given that phytase supplementation in broiler diets is commonplace, understanding possible synergistic or antagonistic relationships between phytase, CB, and BMD is essential for improving intestinal health and performance. Results obtained when investigating effects of MMFA with a certain inclusion of phytase cannot reliably be extrapolated to other inclusion concentrations.

FUNDING SOURCE

This research was funded by a USDA-ARS Cooperative Agreement (G00014614 - Dietary manipulations to improve gut health in broiler production).

ACKNOWLEDGEMENTS

The authors are grateful for the graduate students, undergraduate students, and visiting research scholars from Drs. Hauck, Pacheco, Dozier, and Macklin labs for their assistance during this experiment. Additionally, the authors are grateful for the Auburn University Charles. C. Miller Jr. Poultry Research Center and Poultry and Animal Nutrition Center staff for their constant support and contributions during each experiment.

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Table 4.1. The primer sequences used for expression of targeted genes using quantitative PCR.

Target gene	Primer sequences (5’-3’)[1]	PCR efficiency	Amplicon length (base pairs)	Reference
Claudin 1 (CL-1)	F: CTGATTGCTTCCAACCAG R: CAGGTCAAACAGAGGTACAAG	1.86	140	García et al., 2021
Claudin 2 (CL-2)	F: CCTCAGCCCTCCATCAAA R: CTGCGTCTTCTCCTCTTACTGT	2.04	162	Ozden et al., 2010
Claudin 4 (CL-4)	F: GAAGCGCTGAACCGATACCA R: TGCTTCTGTGCCTCAGTTTCC	1.85	137	Rahner et al., 2004
Claudin 5 (CL-5)	F: CATCACTTCTCCTTCGTCAGC R: GCACAAAGATCTCCCAGGTC	1.95	224	García et al., 2021
Zonula occludens 1 (ZO-1)	F: CTTCAGGTGTTTCTCTTCCTCCTC R: CTGTGGTTTCATGGCTGGATC	1.98	101	Hunziker et al., 2009
Zonula occludens 2 (ZO-2)	F: CGGCAGCTATCAGACCACTC R: CACAGACCAGCAAGCCTACAG	1.98	89	Honkatukia et al., 2011
Mucin 2 (MUC-2)	F: GCCTGCCCAGGAAATCAAG R: CGACAAGTTTGCTGGCACAT	1.97	59	Wang et al., 2019
GAPDH	F: TGGAGAAACCAGCCAAGTAT R: GCATCAAAGGTGGAGGAAT	2.07	145	Wang et al., 2018
HMBS	F: GATGGATCCGATAGCCTGAA R: GATGTGCTTAGCTCCCTTGC	2.01	195	Zhang et al., 2018

[1] F = Forward; R = Reverse.

Table 4.2. Independent orthogonal and orthogonal polynomial contrasts[1] for intestinal permeability and jejunal villus height (VH), crypt depth (CD), and their ratio (VH:CD) between YPM x Ross 708 male broilers provided a negative control (NC; 0 FTU/kg) diet and a NC with phytase supplementation (500 or 1,500 FTU/kg) from 1 to 42 d of age[2].

	d 27	d 28			d 42		
Item	FITC-d[3], mg/mL	VH, µm	CD, µm	VH:CD ratio	VH, µm	CD, µm	VH:CD
NC vs. NC + phytase[4]							
NC	0.0047	966	204	4.86	1,101	192	5.84
NC + 500 FTU/kg	0.0047	973	201	5.01	1,085	170	6.56
NC + 1,500 FTU/kg	0.0047	986	212	4.79	1,071	170	6.51
CLM[5]	± 0.0006	± 58	± 15	± 0.35	± 66	± 11	± 0.47
	P-value						
NC vs. NC + 500 FTU/kg	0.968	0.866	0.809	0.522	0.731	0.008	0.032
NC vs. NC + 1,500 FTU/kg	0.957	0.638	0.436	0.799	0.525	0.008	0.047
Linear	0.960	0.633	0.367	0.677	0.536	0.018	0.089
Quadratic	0.981	0.990	0.569	0.412	0.882	0.045	0.091

[1] Orthogonal polynomial contrasts were performed for the unequally spaced phytase concentrations (0, 500, and 1,500 FTU/kg).

[2] Intestinal permeability: values are least square means of 8 replicate pens, with each pen having blood collected from 2 broilers on d 27; Jejunum histomorphometry: values are least square means of 8 replicate pens, with each pen having jejunum samples collected from 5 broilers (duplicated; n = 10/pen) on d 28 and 42; statistical significance was considered at $P \leq 0.05$.

[3] Intestinal permeability was assessed by determining the amount of fluorescein isothiocyanate dextran (FITC-d) concentration in the blood.

[4] OptiPhos Plus (Huvepharma Inc., Peachtree City, GA) provided 500 FTU/kg or 1,500 FTU/kg of phytase activity per kg of diet.

[5] CLM = 95% confidence limit for the mean.

Table 4.3. Intestinal permeability and jejunal villus height (VH), crypt depth (CD), and their ratio (VH:CD) of YPM x Ross 708 male broilers provided a negative control diet varying in phytase, calcium butyrate (CB), and bacitracin methylene disalicylate (BMD) inclusion from 1 to 42 d of age[1].

		d 27	d 28			d 42		
Item		FITC-d[2], mg/mL	VH, µm	CD, µm	VH:CD ratio	VH, µm	CD, µm	VH:CD
Phytase[3], FTU/kg, main effect (n = 32)								
500		0.0048	1,013	207	5.03	1,074	167	6.60
1,500		0.0049	991	199	5.11	1,074	167	6.57
CLM[4]		± 0.0002	± 30	± 7	± 0.17	± 32	± 6	± 0.24
Microbiota modulating feed additives, main effect (n = 16)								
None		0.0047	979	207	4.90	1,078^{ab}	170	6.53
Only CB[5]		0.0050	1,011	207	4.99	1,142^{a}	170	6.85
Only BMD[6]		0.0048	1,002	199	5.17	1,042^{b}	171	6.27
Both CB and BMD		0.0050	1,016	199	5.21	1,033^{b}	158	6.70
CLM		± 0.0003	± 43	± 10	± 0.24	± 45	± 8	± 0.34
Phytase, FTU/kg x	Microbiota modulating feed additives							
500	None	0.0047	973^{ab}	201	5.01	1,085	170	6.56
500	Only CB	0.0049	995^{ab}	217	4.67	1,132	170	6.80
500	Only BMD	0.0047	1,001^{ab}	202	5.08	1,062	174	6.30
500	Both CB and BMD	0.0051	1,083^{a}	207	5.36	1,016	154	6.76
1,500	None	0.0047	986^{ab}	212	4.79	1,071	170	6.51
1,500	Only CB	0.0051	1,028^{ab}	197	5.31	1,153	170	6.90
1,500	Only BMD	0.0050	1,003^{ab}	197	5.27	1,021	169	6.24
1,500	Both CB and BMD	0.0050	948^{b}	191	5.07	1,049	161	6.65
CLM		± 0.0005	± 60	± 15	± 0.34	± 63	± 11	± 0.48
					P-value			
Phytase		0.457	0.305	0.157	0.512	0.999	0.943	0.867
Microbiota modulating feed additives		0.453	0.633	0.552	0.224	0.004	0.070	0.106
Phytase x Microbiota modulating feed additives		0.611	0.030	0.184	0.029[†]	0.627	0.728	0.975

[a,b] Means within a column with different superscripts differ significantly ($P \leq 0.05$).

[1] Intestinal permeability: interaction values are least square means of 8 replicate pens, with each pen having blood collected from 2 broilers on d 27; Jejunum histomorphometry: interaction values are least square means of 8 replicate pens, with each pen having jejunum samples collected from 5 broilers (duplicated; n = 10/pen) on d 28 and 42.

[2] Intestinal permeability was assessed by determining the amount of fluorescein isothiocyanate dextran (FITC-d) concentration in the blood.

[3] OptiPhos Plus (Huvepharma Inc., Peachtree City, GA) provided 500 FTU/kg or 1,500 FTU/kg of phytase activity per kg of diet.

[4] CLM = 95% confidence limit for the mean.

[5] ButiPEARL (Kemin Industries Inc., Des Moines, IA) included at 0 or 0.5 g supplement/kg of diet.

[6] Bacitracin methylene disalicylate 50 Type A Medicated Article (Zoetis, Parsippany, NJ) included at 0 or 55 mg per kg of diet.

[†] Overall interaction was significant; however, Tukey-Kramer multiple comparison test did not separate the means.

Table 4.4. Independent orthogonal and orthogonal polynomial contrasts[1] for tight junction (CL-1, CL-2, CL-4, CL-5, ZO-1, and ZO-2) and mucin (MUC-2) gene expression between YPM x Ross 708 male broilers provided a negative control (NC; 0 FTU/kg) diet and a NC with phytase supplementation (500 or 1,500 FTU/kg) on d 28[2].

	Gene expression, Log2						
Item	CL-1	CL-2	CL-4	CL-5	ZO-1	ZO-2	MUC-2
NC vs. NC + phytase[3]							
NC	-0.278	0.171	-1.387	-0.095	0.083	0.085	0.357
NC + 500 FTU/kg	-0.436	-0.650	-0.990	-0.043	0.297	0.071	0.825
NC + 1,500 FTU/kg	-1.004	-0.340	-1.330	-0.507	0.010	0.048	1.052
CLM[4]	± 0.392	± 0.561	± 1.128	± 0.434	± 0.291	± 0.349	± 0.774
	P-value						
NC vs. NC + 500 FTU/kg	0.563	0.043	0.612	0.867	0.303	0.954	0.373
NC vs. NC + 1,500 FTU/kg	0.008	0.204	0.941	0.185	0.727	0.881	0.199
Linear	0.005	0.345	0.976	0.139	0.545	0.880	0.227
Quadratic	0.728	0.068	0.580	0.489	0.195	0.993	0.611

[1] Orthogonal polynomial contrasts were performed for the unequally spaced phytase concentrations (0, 500, and 1,500 FTU/kg).

[2] Values are least square means of 10 broilers per treatment, with each pen having jejunum samples collected on d 28; statistical significance was considered at $P \leq 0.05$.

[3] OptiPhos Plus (Huvepharma Inc., Peachtree City, GA) provided 500 FTU/kg or 1,500 FTU/kg of phytase activity per kg of diet.

[4] CLM = 95% confidence limit for the mean.

Table 4.5. Tight junction (CL-1, CL-2, CL-4, CL-5, ZO-1, and ZO-2) and mucin (MUC-2) gene expression of YPM x Ross 708 male broilers provided a negative control diet varying in phytase, calcium butyrate (CB), and bacitracin methylene disalicylate (BMD) inclusion on d 28[1].

		Gene expression, Log2						
Item		CL-1	CL-2	CL-4	CL-5	ZO-1	ZO-2	MUC-2
Phytase[2], FTU/kg, main effect (n = 40)								
500		-0.708	0.059	-1.456	-0.242	0.188	0.012	0.739
1,500		0.020	-0.229	1.346	0.075	0.338	0.766	0.806
CLM[3]		± 0.197	± 0.269	± 0.568	± 0.228	± 0.159	± 0.175	± 0.364
Microbiota modulating feed additives, main effect (n = 20)								
None		-0.720	-0.495^{b}	-1.160	-0.275	0.154	0.059	0.938
Only CB[4]		0.431	-0.081^{ab}	2.237	-0.008	0.094	1.203	0.918
Only BMD[5]		-0.435	0.228^{a}	-1.218	-0.147	0.435	0.156	0.734
Both CB and BMD		-0.652	-0.052^{ab}	-0.078	0.094	0.370	0.138	0.500
CLM		± 0.286	± 0.391	± 0.850	± 0.327	± 0.232	± 0.270	± 0.529
Phytase, FTU/kg x	Microbiota modulating feed additives							
500	None	-0.436^{bc}	-0.650	-0.990^{b}	-0.043^{ab}	0.297	0.071^{b}	0.825
500	Only CB	-1.007^{c}	0.310	-2.140^{b}	-0.545^{b}	-0.039	0.077^{b}	1.410
500	Only BMD	-0.791^{bc}	0.313	-2.243^{b}	-0.336^{ab}	0.169	-0.125^{b}	0.251
500	Both CB and BMD	-0.598^{bc}	0.264	-0.450^{b}	-0.045^{ab}	0.326	0.026^{b}	0.471
1,500	None	-1.004^{c}	-0.340	-1.330^{b}	-0.507^{b}	0.010	0.048^{b}	1.052
1,500	Only CB	1.869^{a}	-0.473	6.614^{a}	0.529^{a}	0.226	2.329^{a}	0.426
1,500	Only BMD	-0.078^{b}	0.263	-0.192^{b}	0.042^{ab}	0.701	0.436^{b}	1.218
1,500	Both CB and BMD	-0.706^{bc}	-0.367	0.294^{b}	0.234^{ab}	0.414	0.249^{b}	0.529
CLM		± 0.429	± 0.569	± 1.275	± 0.488	± 0.337	± 0.395	± 0.770
		P-value						
Phytase		<0.001	0.127	<0.001	0.051	0.187	<0.001	0.792
Microbiota modulating feed additives		<0.001	0.033	<0.001	0.366	0.101	<0.001	0.587
Phytase x Microbiota modulating feed additives		<0.001	0.144	<0.001[†]	0.012	0.066	<0.001	0.069

[a-c] Means within a column with different superscripts differ significantly ($P \le 0.05$).

[1] Interaction values are least square means of 10 broilers per treatment, with each pen having jejunum samples collected on d 28.

[2] OptiPhos Plus (Huvepharma Inc., Peachtree City, GA) provided 500 FTU/kg or 1,500 FTU/kg of phytase activity per kg of diet.

[3] CLM = 95% confidence limit for the mean.

[4] ButiPEARL (Kemin Industries Inc., Des Moines, IA) included at 0 or 0.5 g supplement/kg of diet.

[5] Bacitracin methylene disalicylate 50 Type A Medicated Article (Zoetis, Parsippany, NJ) included at 0 or 55 mg per kg of diet.

[†] Conservative Tukey-Kramer grouping for interaction.

Table 4.6. Independent orthogonal and orthogonal polynomial contrasts[1] for tight junction (CL-1, CL-2, CL-4, CL-5, ZO-1, and ZO-2) and mucin (MUC-2) gene expression between YPM x Ross 708 male broilers provided a negative control (NC; 0 FTU/kg) diet and a NC with phytase supplementation (500 or 1,500 FTU/kg) on d 42[2].

	Gene expression, Log2						
Item	CL-1	CL-2	CL-4	CL-5	ZO-1	ZO-2	MUC-2
NC vs. NC + phytase[3]							
NC	-0.295	-0.183	-1.216	0.004	-0.110	0.123	0.391
NC + 500 FTU/kg	-0.395	-0.517	-0.714	-0.093	-0.071	-0.335	0.292
NC + 1,500 FTU/kg	-0.507	-1.943	-2.331	0.090	-0.161	-0.762	-1.123
CLM[4]	± 0.269	± 0.805	± 1.597	± 0.405	± 0.284	± 0.292	± 0.650
	P-value						
NC vs. NC + 500 FTU/kg	0.602	0.527	0.642	0.735	0.847	0.022	0.823
NC vs. NC + 1,500 FTU/kg	0.258	0.002	0.315	0.767	0.797	<0.001	<0.001
Linear	0.261	0.001	0.229	0.692	0.745	<0.001	<0.001
Quadratic	0.862	0.585	0.350	0.620	0.753	0.355	0.285

[1] Orthogonal polynomial contrasts were performed for the unequally spaced phytase concentrations (0, 500, and 1,500 FTU/kg).

[2] Values are least square means of 10 broilers per treatment, with each pen having jejunum samples collected on d 42; statistical significance was considered at $P \leq 0.05$.

[3] OptiPhos Plus (Huvepharma Inc., Peachtree City, GA) provided 500 FTU/kg or 1,500 FTU/kg of phytase activity per kg of diet.

[4] CLM = 95% confidence limit for the mean.

Table 4.7. Tight junction (CL-1, CL-2, CL-4, CL-5, ZO-1, and ZO-2) and mucin (MUC-2) gene expression of YPM x Ross 708 male broilers provided a negative control diet varying in phytase, calcium butyrate (CB), and bacitracin methylene disalicylate (BMD) inclusion on d 42[1].

Item		Gene expression, Log2						
		CL-1	CL-2	CL-4	CL-5	ZO-1	ZO-2	MUC-2
Phytase[2], FTU/kg, main effect (n = 40)								
500		-0.248	-0.111	-0.711	0.026	0.029	-0.258	0.134^{a}
1,500		-0.365	-0.899	-0.191	0.181	-0.002	-0.275	-0.430^{b}
CLM[3]		± 0.134	± 0.368	± 0.775	± 0.220	± 0.144	± 0.133	± 0.317
Microbiota modulating feed additives, main effect (n = 20)								
None		-0.451^{b}	-1.230	-1.522	-0.002	-0.116	-0.548^{b}	-0.415^{b}
Only CB[4]		-0.385^{b}	-0.012	-0.642	-0.002	0.042	-0.163^{a}	0.449^{a}
Only BMD[5]		-0.393^{b}	-0.255	-0.053	0.063	-0.109	-0.180^{a}	-0.362^{ab}
Both CB and BMD		0.003^{a}	-0.522	0.414	0.353	0.236	-0.175^{a}	-0.265^{ab}
CLM		± 0.192	± 0.536	± 1.082	± 0.321	± 0.206	± 0.190	± 0.456
Phytase, FTU/kg x	Microbiota modulating feed additives							
500	None	-0.395	-0.517^{abc}	-0.714	-0.093	-0.071	-0.335	0.292
500	Only CB	-0.323	0.065^{ab}	-0.853	0.149	0.099	-0.212	0.854
500	Only BMD	-0.206	0.590^{a}	-0.701	-0.351	-0.280	-0.275	-0.390
500	Both CB and BMD	-0.067	-0.580^{abc}	-0.576	0.398	0.366	-0.211	-0.223
1,500	None	-0.507	-1.943^{c}	-2.331	0.090	-0.161	-0.762	-1.123
1,500	Only CB	-0.447	-0.090^{ab}	-0.432	-0.152	-0.016	-0.115	0.044
1,500	Only BMD	-0.580	-1.100^{bc}	0.595	0.476	0.062	-0.086	-0.334
1,500	Both CB and BMD	0.073	-0.464^{abc}	1.404	0.309	0.105	-0.138	-0.307
CLM		± 0.271	± 0.799	± 1.570	± 0.493	± 0.291	± 0.292	± 0.680
		P-value						
Phytase		0.217	0.004	0.341	0.308	0.759	0.855	0.013
Microbiota modulating feed additives		0.005	0.012	0.078	0.293	0.051	0.011	0.028
Phytase x Microbiota modulating feed additives		0.309	0.035	0.112	0.056	0.169	0.096	0.068

[a-c] Means within a column with different superscripts differ significantly ($P \leq 0.05$).

[1] Interaction values are least square means of 10 broilers per treatment, with each pen having jejunum samples collected on d 42.

[2] OptiPhos Plus (Huvepharma Inc., Peachtree City, GA) provided 500 FTU/kg or 1,500 FTU/kg of phytase activity per kg of diet.

[3] CLM = 95% confidence limit for the mean.

[4] ButiPEARL (Kemin Industries Inc., Des Moines, IA) included at 0 or 0.5 g supplement/kg of diet.

[5] Bacitracin methylene disalicylate 50 Type A Medicated Article (Zoetis, Parsippany, NJ) included at 0 or 55 mg per kg of diet.

Table 4.8. Independent orthogonal and orthogonal polynomial contrasts[1] for cecal microbiome alpha diversity characteristics between YPM x Ross 708 male broilers provided a negative control (NC; 0 FTU/kg) diet and a NC with phytase supplementation (500 or 1,500 FTU/kg) on d 28 and 42[2].

	d 28			d 42		
Item	Evenness[3]	FPD[3]	OF[3]	Evenness	FPD	OF
NC vs. NC + phytase[4]						
NC	0.785	14.52	322	0.766	17.36	441
NC + 500 FTU/kg	0.760	14.33	330	0.778	18.20	461
NC + 1,500 FTU/kg	0.799	14.91	360	0.747	17.25	398
CLM[5]	± 0.020	± 0.89	± 32	± 0.023	± 1.15	± 43
	P-value					
NC vs. NC + 500 FTU/kg	0.072	0.734	0.682	0.430	0.275	0.464
NC vs. NC + 1,500 FTU/kg	0.326	0.504	0.080	0.196	0.888	0.145
Linear	0.144	0.432	0.070	0.109	0.689	0.079
Quadratic	0.017	0.518	0.820	0.155	0.184	0.153

[1] Orthogonal polynomial contrasts were performed for the unequally spaced phytase concentrations (0, 500, and 1,500 FTU/kg).

[2] Values are least square means of 8 replicate pens, with each pen having cecal samples collected on d 28 and 42; statistical significance was considered at $P \leq 0.05$.

[3] Evenness = Pielou's evenness; FPD = Faith's phylogenetic diversity; OF = Observed features.

[4] OptiPhos Plus (Huvepharma Inc., Peachtree City, GA) provided 500 FTU/kg or 1,500 FTU/kg of phytase activity per kg of diet.

[5] CLM = 95% confidence limit for the mean.

Table 4.9. Cecal microbiome alpha diversity characteristics of YPM x Ross 708 male broilers provided a negative control diet varying in phytase, calcium butyrate (CB), and bacitracin methylene disalicylate (BMD) inclusion on d 28 and 42[1].

		d 28			d 42		
Item		Evenness[2]	FPD[2]	OF[2]	Evenness	FPD	OF
Phytase[3], FTU/kg, main effect (n = 40)							
500		0.779	15.00	349^{b}	0.765	17.28	426
1,500		0.795	15.41	375^{a}	0.771	17.47	427
CLM[4]		± 0.011	± 0.41	± 16	± 0.010	± 0.52	± 20
Microbiota modulating feed additives, main effect (n = 20)							
None		0.779	14.62	345	0.763	17.72	429
Only CB[5]		0.772	15.49	360	0.767	17.22	422
Only BMD[6]		0.796	15.37	370	0.769	17.71	443
Both CB and BMD		0.800	15.34	372	0.772	16.85	412
CLM		± 0.017	± 0.58	± 23	± 0.015	± 0.75	± 28
Phytase, FTU/kg x	Microbiota modulating feed additives						
500	None	0.760^{bc}	14.33	330	0.778^{ab}	18.20	461
500	Only CB	0.747^{c}	15.22	341	0.761^{ab}	16.73	413
500	Only BMD	0.815^{a}	15.57	373	0.776^{ab}	17.56	443
500	Both CB and BMD	0.795^{abc}	14.87	352	0.743^{b}	16.63	388
1,500	None	0.799^{ab}	14.91	360	0.747^{b}	17.25	398
1,500	Only CB	0.797^{abc}	15.76	379	0.772^{ab}	17.70	430
1,500	Only BMD	0.777^{abc}	15.17	366	0.762^{ab}	17.86	443
1,500	Both CB and BMD	0.806^{ab}	15.81	393	0.802^{a}	17.08	436
CLM		± 0.024	± 0.88	± 33	± 0.022	± 1.06	± 43
				P-value			
Phytase		0.051	0.146	0.026	0.401	0.598	0.973
Microbiota modulating feed additives		0.042	0.149	0.310	0.829	0.278	0.440
Phytase x Microbiota modulating feed additives		<0.001	0.365	0.417	<0.001	0.285	0.040[†]

[a-c]Means within a column with different superscripts differ significantly ($P \leq 0.05$).

[1] Interaction values are least square means of 8 replicate pens, with each pen having cecal samples collected on d 28 and 42.

[2] Evenness = Pielou's evenness; FPD = Faith's phylogenetic diversity; OF = Observed features.

[3] OptiPhos Plus (Huvepharma Inc., Peachtree City, GA) provided 500 FTU/kg or 1,500 FTU/kg of phytase activity per kg of diet.

[4] CLM = 95% confidence limit for the mean.

[5] ButiPEARL (Kemin Industries Inc., Des Moines, IA) included at 0 or 0.5 g supplement/kg of diet.

[6] Bacitracin methylene disalicylate 50 Type A Medicated Article (Zoetis, Parsippany, NJ) included at 0 or 55 mg per kg of diet.

[†] Overall interaction was significant; however, Tukey-Kramer multiple comparison test did not separate the means.

Table 4.10. Number of up- and down-regulated differentially expressed genes (DEGs) based on predicted metagenome of the cecal microbiota compared between YPM x Ross 708 male broilers provided a negative control (NC; 0 FTU/kg) diet and a NC with phytase[1] supplementation (500 or 1,500 FTU/kg) on d 28 and 42.

Comparison	d 28		d 42		Unique DEGs	Intersections[2]
	Up	Down	Up	Down		
NC + 500 FTU/kg vs. NC	16	87	7	1,333	1,439	2
NC + 1,500 FTU/kg vs. NC	100	84	0	4	188	0

[1] OptiPhos Plus (Huvepharma Inc., Peachtree City, GA).

[2] Intersections indicate the common DEGs observed on both d 28 and 42.

Table 4.11. Number of up- and down-regulated differentially expressed genes (DEGs) based on predicted metagenome of the cecal microbiota in YPM x Ross 708 male broilers provided a negative control diet varying in phytase[1], calcium butyrate[2] (CB), and bacitracin methylene disalicylate[3] (BMD) inclusion on d 28 and 42.

Comparison	d 28		d 42		Unique DEGs	Intersections[4]
	Up	Down	Up	Down		
Phytase[5]						
1,500 vs. 500 FTU/kg	0	4	0	0	4	0
MMFA[6]						
All other MMFA[7] vs. None	3	0	0	0	3	0
Only CB vs. None	0	0	0	0	0	0
Only BMD vs. None	1	6	0	1	8	0
Both CB and BMD vs. None	0	4	35	8	47	0

[1] OptiPhos Plus (Huvepharma Inc., Peachtree City, GA).

[2] ButiPEARL (Kemin Industries Inc., Des Moines, IA) included at 0 or 0.5 g supplement/kg of diet.

[3] BMD 50 Type A Medicated Article (Zoetis, Parsippany, NJ) included at 0 or 55 mg per kg of diet.

[4] Intersections indicate the common DEGs observed on both d 28 and 42.

[5] Phytase (FTU/kg) includes: (1) 500 and (2) 1,500.

[6] Microbiota modulating feed additives includes: (1) none, (2), only CB, (3) only BMD, and (4) both CB and BMD.

[7] All other MMFA includes: (2) only CB, (3) only BMD, and (4) both CB and BMD.

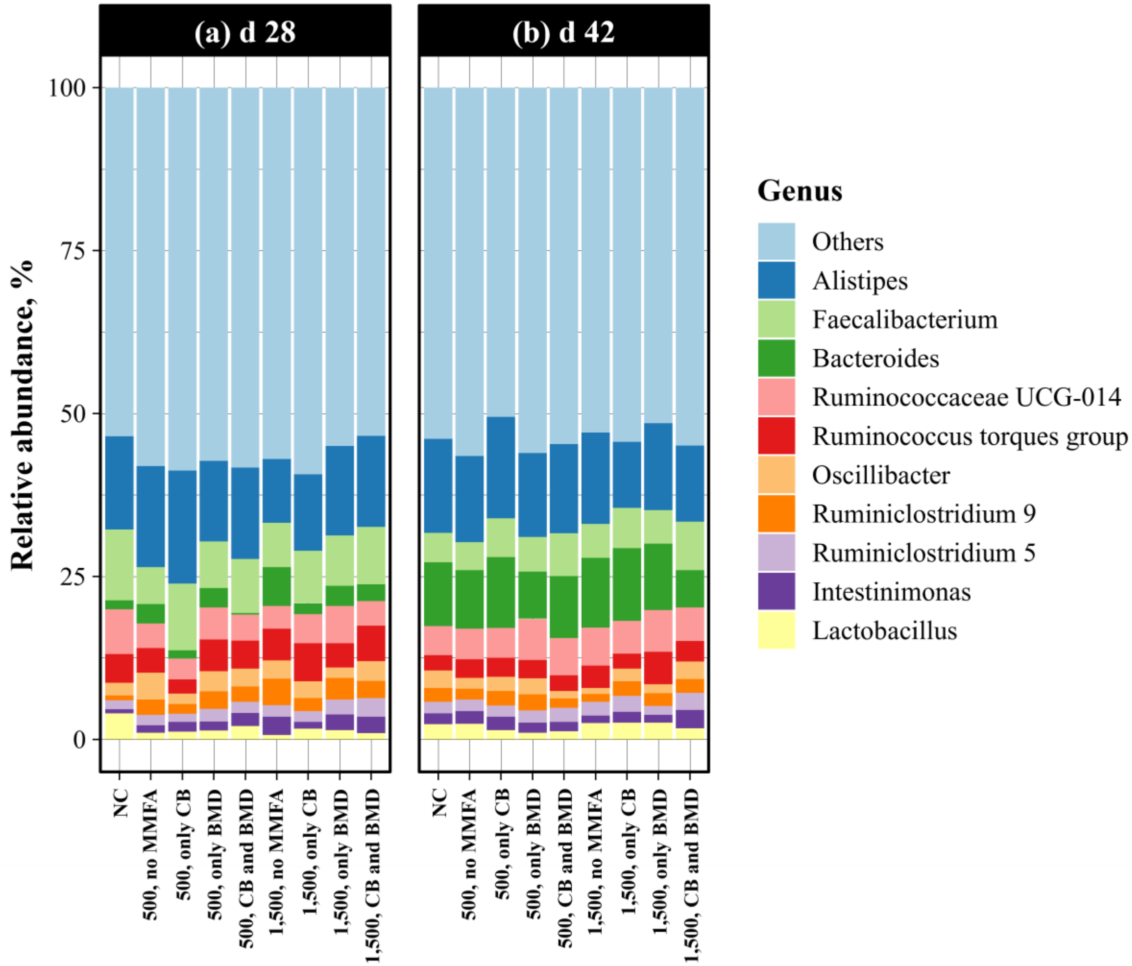

Figure 4.1. Relative abundance of the 10 most abundant genera in the cecal microbiome of YPM x Ross 708 male broilers provided a negative control (NC) diet varying in phytase (500 or 1,500 FTU/kg) and microbiota modulating feed additive inclusion (MMFA; none, only calcium butyrate (CB), only bacitracin methylene disalicylate (BMD), or both CB and BMD) on (**a**) d 28 and (**b**) d 42.

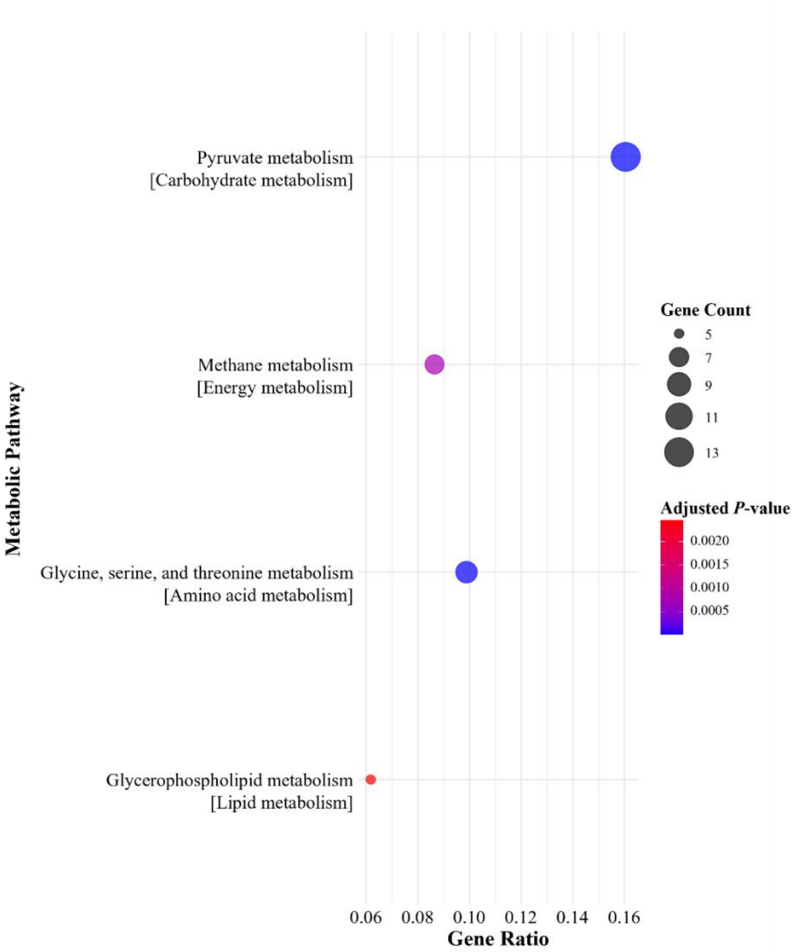

Figure 4.2. Downregulated metabolic pathways based on metagenome predicted from 16S rRNA gene abundances between YPM x Ross 708 male broilers provided a negative control (NC) with 500 FTU/kg of phytase[1] and a NC (0 FTU/kg) diet on d 28. [1]OptiPhos Plus (Huvepharma Inc., Peachtree City, GA).

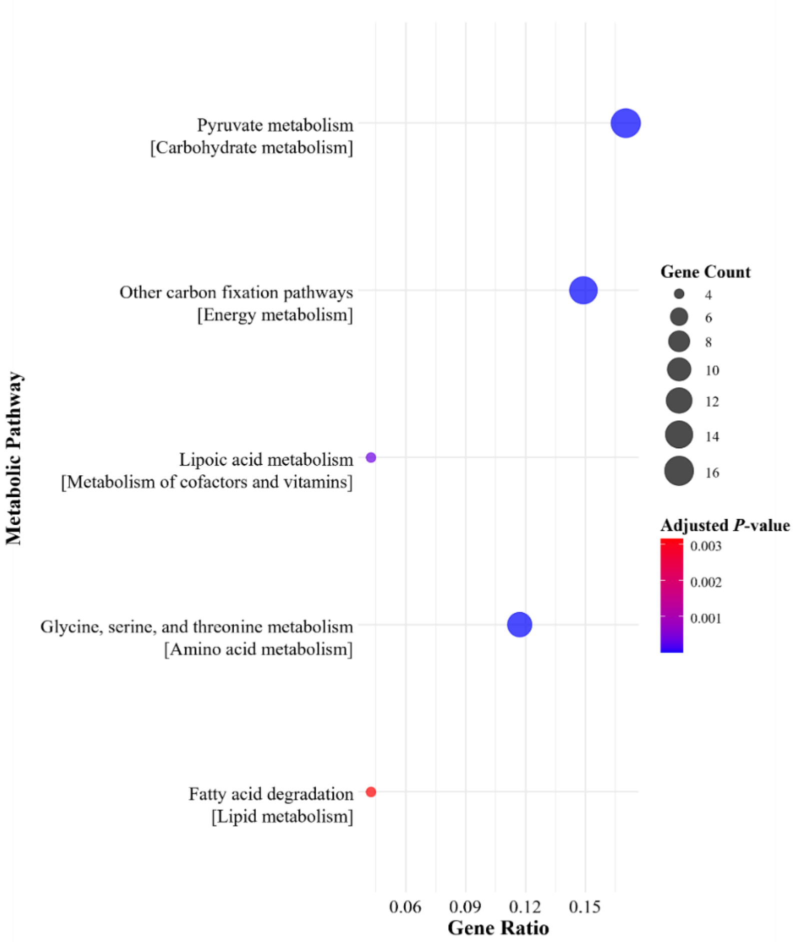

Figure 4.3. Upregulated metabolic pathways based on metagenome predicted from 16S rRNA gene abundances between YPM x Ross 708 male broilers provided a negative control (NC) with 1,500 FTU/kg of phytase[1] and a NC (0 FTU/kg) diet on d 28. [1]OptiPhos Plus (Huvepharma Inc., Peachtree City, GA).

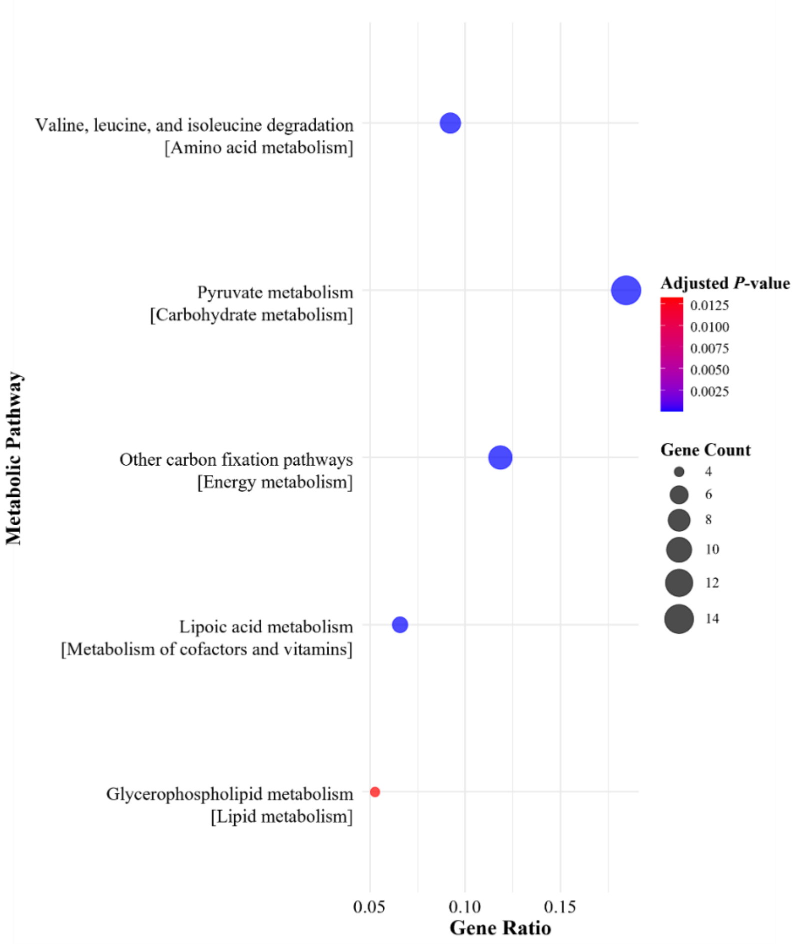

Figure 4.4. Downregulated metabolic pathways based on metagenome predicted from 16S rRNA gene abundances between YPM x Ross 708 male broilers provided a negative control (NC) with 1,500 FTU/kg of phytase[1] and a NC (0 FTU/kg) diet on d 28. [1]OptiPhos Plus (Huvepharma Inc., Peachtree City, GA).

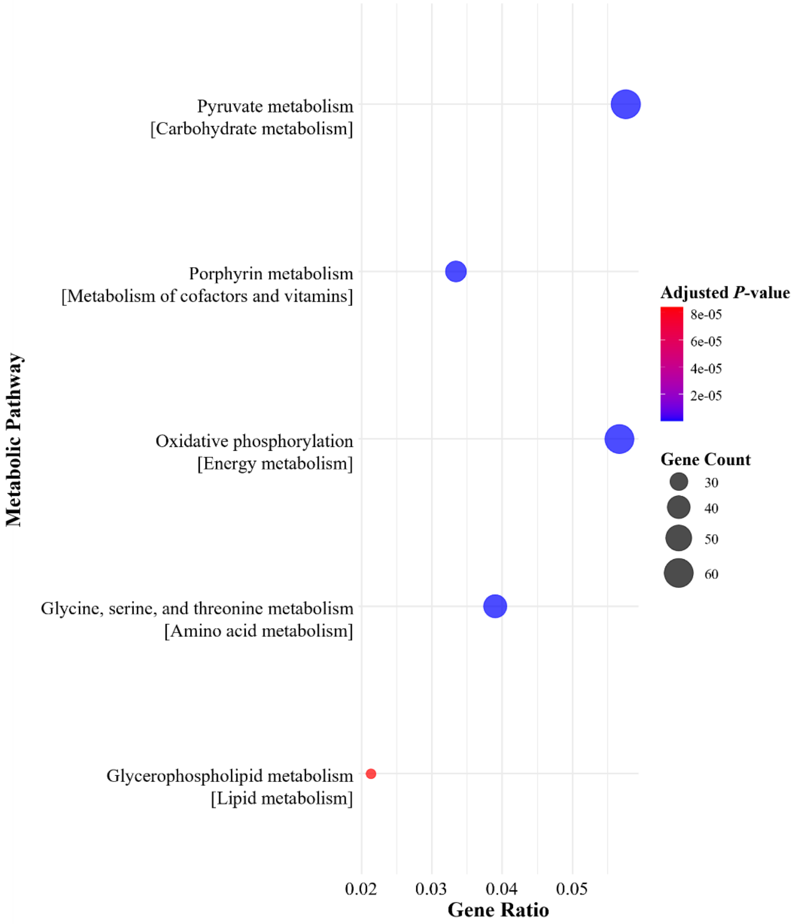

Figure 4.5. Downregulated metabolic pathways based on metagenome predicted from 16S rRNA gene abundances between YPM x Ross 708 male broilers provided a negative control (NC) with 500 FTU/kg of phytase[1] and a NC (0 FTU/kg) diet on d 42. [1]OptiPhos Plus (Huvepharma Inc., Peachtree City, GA).

V. RESPONSE OF BROILERS SUBJECTED TO AN ENTERIC CHALLENGE AND FED DIETS WITH VARYING LIMESTONE PARTICLE SIZES AND CALCIUM CONCENTRATIONS–PART 1: PERFORMANCE, TIBIA MINERALIZATION, AND NUTRIENT DIGESTIBILITY

ABSTRACT

This 35-d experiment evaluated the effects of limestone particle size and calcium concentrations on broiler performance, tibia mineralization, and nutrient digestibility. Two thousand one hundred d-old YPM x 708 male broilers were distributed into 70 floor pens and assigned to 1 of 7 treatments (10 replicates/treatment). This experiment was a 2 x 3 + 1 factorial arrangement, including 2 limestone particle sizes (910 and 200 µm) and 3 calcium concentrations (adequate, reduced, and low), and an unchallenged control. Dietary calcium was reduced in two 0.10 percentage unit steps from adequate concentrations (i.e., breeder recommendations). All factorial treatments were enterically challenged with *Eimeria* spp. and *Clostridium perfringens*. Performance (d 17, 21, 26, and 35), tibia mineralization (d 21 and 35), and nutrient and energy digestibility (d 21 and 35) were measured. The enteric challenge reduced BW and increased feed conversion ratios (**FCR**; contrast, $P \leq 0.05$). Broilers fed adequate and reduced calcium diets had higher BW, tibia shear strength, and tibia ash on d 35 compared to low calcium diets (main effect, $P \leq 0.05$). Day 1 to 35 FCR linearly increased as dietary calcium decreased from adequate to low in the group fed 910 µm limestone, whereas in the 200 µm limestone group, only the low calcium and not the reduced calcium led to higher FCR compared with those fed adequate calcium (interaction, $P \leq 0.05$). Broilers fed reduced and low calcium diets with 910 µm limestone had the highest calcium and phosphorus digestibility on d 21. With 200 μm limestone, broilers fed a low calcium diet had higher mineral digestibility (interaction, $P \leq 0.05$). Day 35 apparent ileal digestible energy (**AIDE**) increased when broilers consumed an adequate or reduced calcium diet compared to low calcium (main effect, $P \leq 0.05$). This experiment demonstrated dietary calcium concentrations 0.10 percentage units lower than recommended concentrations can maintain broiler performance, tibia mineralization, and AIDE during an enteric challenge. However, calcium concentration effects depended on limestone particle size.

INTRODUCTION

Antibiotic growth promotors (**AGP**) have been used to improve poultry production efficiency, weight gain, and feed conversion (Miles et al., 2006; Maria Cardinal et al., 2019). However, AGP usage has declined in recent years, which may result in reduced broiler performance and higher incidence of diseases such as coccidiosis and necrotic enteritis (**NE**) (Engster et al., 2002; Cervantes, 2015). Necrotic enteritis is a multifactorial enteric disease caused by *Clostridium perfringens*, particularly after birds have been predisposed by *Eimeria* infection (Emami and Dalloul, 2021). Various nutritional strategies aimed at improving broiler performance and health without AGP have been reviewed to mitigate the severity of these enteric diseases (Dahiya et al., 2006; Ayalew et al., 2022). These nutritional strategies primarily rely on feed additives (e.g., organic acids and probiotics), however, controlling dietary calcium concentrations and particle size of the calcium source could be a viable strategy to mitigate enteric disease severity.

Calcium is an important macro mineral in broiler nutrition, serving as a structural component of bones, intracellular messenger, and cofactor of various enzymes

(Proszkowiec-Weglarz and Angel, 2013). While adequate calcium is essential for normal development and physiological processes, broiler diets containing high calcium concentrations or highly soluble calcium sources could negatively impact growth and health, particularly under NE challenge conditions (Paiva et al., 2013; Paiva et al., 2014). Due to the low cost and widespread availability of commonly used calcium sources (David et al., 2023), excessive inclusion of calcium in broiler diets is common, emphasizing the need for stricter control over its concentration (Walk, 2016; Li et al., 2017).

High calcium concentrations may contribute to NE pathogenicity through multiple mechanisms. It can promote the synthesis and activity of NetB and α-toxin produced by *C. perfringens*, which disrupts the intestinal mucosa (Keyburn et al., 2010; Fathima et al., 2022). Furthermore, high calcium concentrations can increase calcium-phytate complex formation (Selle et al., 2009), resulting in undigested nutrients that can serve as substrates for *C. perfringens* (Moran, 2014). Beyond dietary calcium concentration, solubility of the calcium source may also influence NE severity (Paiva et al., 2013).

Limestone is a commonly used calcium source in broiler diets worldwide, but its characteristics vary significantly across regions (Gilani et al., 2022). Variation in limestone characteristics such as particle size can influence its solubility, with smaller particles resulting in higher *in vitro* solubility (de Witt et al., 2006; Kim et al., 2019), but a lower *in vivo* solubility (de Witt et al., 2006). When comparing particulate (402 µm) and pulverized limestone (< 75 µm), Kim et al. (2018) reported that pulverized limestone negatively impacted apparent ileal digestibility (**AID**) of calcium and phosphorus (**P**).

Furthermore, Paiva et al. (2013 and 2014) reported a negative impact of higher dietary calcium concentrations and calcium solubility on broiler growth during a naturally occurring clinical NE challenge. Zanu et al. (2020a) expanded on this by examining dietary calcium and phytase concentrations during a subclinical NE challenge, finding that higher dietary calcium combined with the NE challenge reduced CP, carbon, potassium, and zinc digestibility. However, there is still limited data on the response of broilers fed varying limestone particle size and calcium concentrations under subclinical NE conditions. Therefore, the objective of the current experiment was to challenge broilers with *Eimeria* spp. and *C. perfringens* and evaluate the interactive effects of limestone particle size and calcium concentrations on performance, tibia mineralization, and nutrient digestibility.

MATERIALS AND METHODS

Animal Care

This experiment was conducted at the Auburn University Charles C. Miller Jr. Poultry Research and Education Center. All research procedures were approved by the Auburn University Institutional Biosafety Committee (BUA # 984) and Institutional Animal Care and Use Committee (PRN 2023-5159).

Bird Management

Two thousand one hundred male d-old broiler chicks (YPM x Ross 708; Aviagen North America, Huntsville, AL) were obtained from a commercial hatchery. Upon arrival, chicks were weighed and distributed randomly among 70 floor pens (30 birds/pen, 0.09 m^2/bird from d 1 to 21; 22 birds/pen, 0.13 m^2/bird from d 22 to 35) in an environmentally controlled house equipped with exhaust fans, stir fans, forced-air heaters, evaporative-cooling pads, and an electronic controller to manage temperature and ventilation. All floor pens contained new pine shavings with minimal addition of used litter from a previous non-challenged flock. Each pen contained nipple drinkers (5 nipples/pen) and a hanging pan feeder. Feed and water were provided *ad libitum*. The lighting program consisted of 23L:1D from 1 to 7 d and 20L:4D from 8 to 35 d. Environmental temperature was maintained at 33°C on d 1 and was gradually reduced to 20°C by 35 d of age.

Feed Formulation, Manufacture, and Experimental Design

All diets were manufactured at the Auburn University Poultry and Animal Nutrition Center and formulated following Aviagen's recommendations for Ross 708 male broilers (Aviagen, 2022) (**Tables 5.1** and **5.2**). However, calcium concentrations were adjusted in a two-step process, with a 0.10 percentage unit reduction for each growth phase (Starter: Adequate (0.95%), Reduced (0.85%), Low (0.75%); Grower: Adequate (0.85%), Reduced (0.75%), Low (0.65%); Finisher: Adequate (0.75%), Reduced (0.65%), Low (0.55%)). To maintain the balance of other nutrients, sand (inert filler) was added in place of limestone to achieve reduced calcium concentrations. Additionally, amino acid (**AA**) density in the starter feed was formulated at 95.5% of the recommended primary breeder's requirements to account for a longer feeding period of the starter phase. Amino acid density in the grower and finisher diets were formulated according to the primary breeder's recommendations.

Broilers were fed a three-phase feeding program consisting of starter (d 1 to 17; crumbles), grower (d 18 to 26; pellets), and finisher diets (d 27 to 35; pellets) and randomly assigned to 1 of 7 treatments with 10 replicates per treatment. This experiment was a 2 x 3 + 1 factorial arrangement including 2 limestone particle sizes (910 and 200 µm) and 3 calcium concentrations (adequate, reduced, and low). The 6 factorially arranged treatments were subjected to an enteric challenge, while the + 1 treatment was an unchallenged control. The unchallenged control birds were fed diets with 200 µm limestone particle size and adequate calcium concentration at each feeding phase. Lastly, all treatments were supplemented with 1,500 FTU/kg of phytase (Quantum Blue 5 G, AB Vista, Marlborough, Wiltshire, UK), which was formulated to provide 0.192 and 0.175% of calcium and non-phytate P, respectively. Feed samples were sent to an ISO 9001 certified laboratory (AB Vista, Plantation, FL) for phytate-P (NIR analysis of feeds and ingredients using online Feed Quality Service) and phytase activity analysis (QuantiPlate Kits for Quantum Blue® ELISA method (AP181)).

Whole corn was ground with a hammermill (Model 11.5 × 38, Roskamp Champion, Waterloo, IA) equipped with a 4.76-mm screen. Phytase and limestone were premixed with 4.54 kg of ground corn using a countertop mixer (Model A-200, The Hobart Mfg. Co., Troy, OH), prior to their addition to the whole batch of feed. Feed ingredients were blended for 150 s (30 s dry cycle and 120 s wet cycle) using a twin shaft mixer (Model 726, Scott Equipment Co., New Prague, MN) to produce the mash diets. Diets were conditioned at 80°C for 40 s and pelleted through a 4.4-mm pellet die with an effective thickness of 45-mm (length/diameter ratio = 10.23) using a pellet mill (Model 1112-4, California Pellet Mill Co., Crawfordsville, IN). Pellets were dried and cooled using a counter-flow pellet cooler (Model CC0909, California Pellet Mill Co., Crawfordsville, IN). Starter feed was crumbled in a crumbler with manual roll adjustment (Model 624SS, California Pellet Mill Co., Crawfordsville, IN).

All limestone used for this experiment was received from a single source (Tucker Milling, LLC, Guntersville, AL). Geometric diameter average ($\mathbf{d_{gw}}$) of particles for the as-received limestone was 2,496 µm. To achieve a particle size of 910 µm (coarse) and 200 µm (fine), as-received limestone was ground using a 2-pair roller mill (Roskamp Champion Series 900-12, California Pellet Mill Co., Crawfordsville, IN). After grinding, limestone particles were separated using a Dura Tap shaker (Advantech, Mentor, OH). Limestone between U.S.A. Standard Test Sieves No. 16 (1,180 µm) and No. 20 (850 µm) were retained for the coarse particle size (910 µm) groups. Whereas limestone between U.S.A. Standard Test Sieves No. 40 (425 µm) and No. 100 (150 µm) were retained for the fine particle size (200 µm) groups. Afterwards, both limestone particle sizes were subjected to particle size analysis using a Dura Tap shaker (Advantech, Mentor, OH) following the ASABE method S319.4 (ASABE, 2008). Duplicate samples of the 910 and 200 µm limestone were sent to an external laboratory (Chemuniqué (Pty) Ltd, Lanseria, South Africa) for nutrient and solubility analysis. Limestone nutrient and solubility analysis was performed using Inductively Coupled Plasma Spectroscopy and methods described by Kim et al. (2019), respectively.

During feed bagging, feed samples were collected at evenly spaced intervals and combined to form a composite sample, which was analyzed in duplicate. Crude protein (combustion analysis (LECO) AOAC Official Method 990.03; AOAC International, 2006), crude fat (by ether extraction, AOAC Official Method 920.39 (A); AOAC International, 2006), minerals (inductively coupled plasma - optical emission spectroscopy (ICP-OES); AOAC Official Method 985.01 (A, B, D) metals and other elements in plants and pet foods; AOAC International, 2006), and complete protein AA profile were analyzed by the University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO). The amino acid profile of the feed was analyzed using high-performance liquid chromatography (AOAC Official Method 982.30 E (a,b,c), chp 45.3.05; AOAC International, 2006). In addition, tryptophan was analyzed using the AOAC Official Method 988.15, chp. 45.4.04 (AOAC International, 2006). Gross energy (**GE**) of the feed was determined at Auburn University (Auburn, AL) using duplicate 0.75 g samples. Analysis was conducted using an adiabatic oxygen bomb calorimeter (model no. 6400, Parr Instruments, Moline, IA) standardized with benzoic acid.

Enteric Challenge

At 14-d post-hatch, all birds in the challenged groups received an oral gavage with a tenfold dose of a commercially available live attenuated trivalent coccidian vaccine (Advent®; Huvepharma, Inc., Maxton, NC) containing *Eimeria acervulina*, *Eimeria maxima*, and *Eimeria tenella*. In contrast, the unchallenged groups received a sham gavage of sterile phosphate-buffered saline (**PBS**) as a placebo. On d 17, feed was changed from starter to grower to induce a disturbance in the intestinal microbiota. On d 18, the *Eimeria* challenged groups were orally gavaged with 1×10^8 colony-forming units (**cfu**) of NetB-negative *C. perfringens*. Concurrently, the unchallenged groups were given an equal volume of sterile brain heart infusion broth medium (Becton, Dickinson and Company, Sparks, MD).

Measurements

Broilers and feed were weighed on d 1 (average chick weight = 46 g), 17, 21, 26, and 35 to determine BW, feed intake (**FI**), and feed conversion ratio (**FCR**). Birds were inspected twice daily and room temperature, bird condition, mortality, and availability of feed and water were monitored during each inspection. The BW of mortalities were used to adjust FCR.

An indigestible marker (0.5% titanium dioxide, **TiO_2**) was added to the grower (18 to 26 d of age) and finisher (27 to 35 d of age) feeds to determine apparent ileal nutrient digestibility. On d 21, 8 birds per pen, and on d 35, 6 birds per pen were selected randomly and euthanized by CO_2 asphyxiation followed by cervical dislocation in accordance with American Veterinary Medical Association Guidelines (AVMA, 2020). Ileal digesta was gently removed starting 2 cm posterior to the Meckel's diverticulum and ending 2 cm anterior to the ileal-cecal junction to evaluate the digestibility of CP, fat, minerals, energy, and AA. While removing the ileal content, distilled water was used to aid in digesta collection. Ileal contents were pooled within individual pens. Pooled ileal digesta samples were stored on ice, transported to the laboratory, and frozen at -20°C until analysis. Thereafter, ileal digesta samples were lyophilized in a Virtis Genesis Pilot Lyophilizer (SP Industries, Warminster, PA) and then ground using an electric coffee grinder (Capresso 560.4 Infinity, Montvale, NJ) on the finest setting (< 0.5-mm).

Dried ileal digesta samples were sent to the University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO) to be analyzed for CP, crude fat, minerals, and AA profile using the methods mentioned previously. Duplicate 0.75 g samples of ileal digesta were analyzed for GE using an adiabatic oxygen bomb calorimeter (model no. 6400, Parr Instruments, Moline, IA) standardized with benzoic acid. Titanium dioxide concentration of feed and ileal digesta was determined through procedures established by Short et al. (1996). Feed (600 mg in quadruplicate) and ileal digesta (200 mg in duplicate) samples were ashed at 580°C and dissolved in 7.4 M sulfuric acid at 250°C. Addition of hydrogen peroxide (30%) produced a yellow/orange color, with color intensity correlating to TiO_2 concentration of the sample. Each sample was analyzed in duplicate by measuring 1 ml of the resulting solution using a UV/Vis spectrophotometer (SpectraMax Plus 384 Absorbance Microplate Reader, Molecular Devices, LLC., San Jose, CA) with absorbance measured at 410 nm. Analyzed TiO_2 concentrations (as-fed basis) for grower feed samples ranged from 0.42 to 0.48% (average CV and recovery for all grower treatments was 4.08 and 91.12%, respectively), whereas finisher feed samples ranged from 0.44 to 0.49% (average CV and recovery for all finisher treatments was 3.49 and 95.25%, respectively). As for ileal digesta samples, d 21 and 35 CV for TiO_2 values in all treatments ranged from 2.10 to 7.04% and 2.38 to 8.17%, respectively. Apparent ileal digestibility of CP, fat, minerals, energy, and AA were determined using an equation adapted from Stein et al. (2007):

$$\text{AID \%} = \left\{ \frac{\left[\left(\frac{\text{Nutrient}}{\text{TiO}_2} \right)_{\text{diet}} - \left(\frac{\text{Nutrient}}{\text{TiO}_2} \right)_{\text{digesta}} \right]}{\left(\frac{\text{Nutrient}}{\text{TiO}_2} \right)_{\text{diet}}} \right\} \text{ x } 100$$

where $\left(\frac{\text{Nutrient}}{\text{TiO}_2} \right)$ represents the ratio of CP, fat, minerals, energy, or AA to TiO_2 in the diet or ileal digesta. Energy digestibility values obtained from this equation were multiplied by the GE content of feed to determine apparent ileal digestible energy (**AIDE**; DM basis) (Gautier and Rochell, 2020). Digestible nutrient intake (**DNI**) was calculated using an equation adapted from Cowieson et al. (2006):

$$\text{DNI} = (\text{CN}_\text{f} \text{ x FI}) \text{ x DCN}$$

where DNI indicates the amount of digestible nutrients consumed, measured in g or kcal; CN_f represents the nutrient's concentration in the feed; FI is the feed intake in g; and DCN is the nutrient's digestibility coefficient, given as a percentage. In the present experiment, DNI was calculated using the grower and finisher feed nutrient concentration, d 17 to 21 and 27 to 35 FI, and the ileal digestibility coefficients determined on d 21 and 35. A detailed account of DNI results were reported (**Appendix 4**, **Tables S5.1-S5.6** and **Figures S5.1-S5.7**).

In addition, on d 21 and 35, the left tibia from 4 birds/pen were excised to determine tibia weight, shear strength, and ash content. Tibias were placed in sample bags, and frozen at -20°C before analysis. All tibias were thawed at room temperature and cleaned. Tissue and cartilaginous caps were removed from each tibia. All clean tibias were weighed prior to shear strength assessment. Shear strength of tibias from d 21 were assessed using a TA.XT plus texture analyzer (Stable Microsystems, Surrey, UK), whereas tibias from d 35 were assessed using a TA.XT plus 100C texture analyzer (Stable Microsystems, Surrey, UK) according to the official method (ANSI/ASAE method S459; ASABE, 2017). Due to load capacity limitations, 2 texture analyzers were needed to assess tibia shear strength at different ages. A test speed of 5 mm/sec and a trigger force of 5 g were used for both texture analyzers. The highest peak was recorded in newtons (**N**) as the maximum force to fracture or break the tibia. After breaking all tibias for shear strength determination, a modified bone ash protocol was followed according to Hall et al. (2003). A 6-positioned heating mantle (HM-200-MP6-1000-115, Cole-Parmer, Vernon Hills, IL) was used to house 6 Soxhlet apparatuses (VWR International, Radnor, PA). Tibias were individually packed using cotton cheesecloth

(Purewipe Cheesecloth, American Fiber and Finishing Inc., Albemarle, NC) and soaked in 200 proof ethanol for 24 h. Subsequently, tibias were placed in one of the Soxhlet apparatuses and exposed to sequential extractions (24 h in 200 proof pure ethanol followed by 24 h in anhydrous ethyl ether). Ethanol was used to remove water and non-polar lipids, whereas anhydrous ether removed polar lipids. Thereafter, tibias were dried at room temperature for a minimum of 24 h and weighed. After weighing, tibias were ashed in a muffle furnace at 580°C. Once ashing was completed, weights were recorded, and tibia ash calculated. Additional samples were collected to evaluate health and physiological measurements, which will be reported in a companion paper (Tabish et al., 2024).

Statistical Analyses

This experiment was a completely randomized design with data analyzed as a 2 x 3 factorial to evaluate the main effects of limestone particle size and calcium concentration and their interactions during an enteric challenge. There were 2 limestone particle sizes (910 and 200 µm) and 3 calcium concentrations (adequate, reduced, and low). The unchallenged control was excluded from this factorial analysis. However, independent orthogonal contrasts were used to compare the unchallenged control with the challenged treatment, both of which contained 200 µm limestone particle size and adequate calcium concentration.

As a main effect, equally spaced calcium concentrations (Starter: Adequate (0.95 %), Reduced (0.85 %), Low (0.75 %); Grower: Adequate (0.85 %), Reduced (0.75 %), Low (0.65 %); Finisher: Adequate (0.75 %), Reduced (0.65 %), Low (0.55 %)) were evaluated using orthogonal polynomial contrasts to assess linear and quadratic trends in the response. In addition, orthogonal polynomial contrasts were performed for the equally spaced calcium concentrations within each limestone particle size group. Only significant calcium concentration main effects and interactions will be accompanied by orthogonal polynomial contrast *P*-values in the text. All orthogonal polynomial contrast *P*-values are detailed in **Appendix 4** (**Tables S5.7-S5.14**).

Each treatment was represented by 10 replicate pens with pen considered as the experimental unit. Percentage data were arcsine-transformed before statistical analysis. Using Tukey's method, outliers were determined when values were 1.5 times the interquartile range below and above the first and third quartile, respectively (Tukey, 1977). All data were analyzed using the GLIMMIX procedure of the SAS software version 9.4 (SAS Institute Inc., Cary, NC). Least square means were compared using Tukey-Kramer with statistical significance considered at $P \leq 0.05$. For all means, 95% confidence limits (**CLM**) were determined. If the Tukey-Kramer test was unable to separate the means, then interactions will be described by determining the overlap of 95% CLM between treatments (Cumming and Fidler, 2009).

RESULTS AND DISCUSSION

The roles of calcium in broiler growth and physiology are well-documented and have been extensively reviewed (Adedokun and Adeola, 2013; Proszkowiec-Weglarz and Angel, 2013; David et al., 2023). However, reports highlighting the common occurrence of excess calcium concentrations in broiler diets (Walk, 2016) have generated interest in moving to a digestible calcium system (Walk et al., 2021). Limiting excess dietary calcium during a NE challenge has been an effective approach to control disease related mortality (Paiva et al., 2014). Concomitantly, limestone particle size has been researched for its importance in layers (Hervo et al., 2022), but research is limited in broilers. This is especially important since limestone particle size is highly variable by global region (Gilani et al., 2022). The present experiment evaluated interactive effects of limestone particle size and calcium concentrations on broiler performance, tibia mineralization, and nutrient digestibility during an enteric challenge with *Eimeria* spp. and *C. perfringens*. Further measurements addressing the influence of these interactions on intestinal health and physiology will be discussed in the companion paper (Tabish et al., 2024).

Feed and Phytase Analysis

Analysis of GE, CP, calcium, total P, crude fat, and AA for the adequate, reduced, and low calcium treatments fed during the starter (0.95, 0.85, and 0.75% calcium), grower (0.85, 0.75, and 0.65% calcium), and finisher (0.75, 0.65, and 0.55% calcium) phases are shown in **Table 5.2**. All nutrient analyses were averaged between treatments with similar calcium concentrations and different limestone particle sizes. All analyzed dietary calcium concentrations were lower than the calculated values. However, incremental reductions from adequate to low calcium concentrations were achieved for the grower and finisher feed, but not for the starter feed. As detailed in the tibia mineralization section, reduced dietary calcium was confirmed by decreased tibia ash, a sensitive indicator of calcium absorption (Walk et al., 2011; Olgun et al., 2024). On average, starter, grower, and finisher diets contained 0.254 (SD = 0.0049%; CV = 1.95%), 0.237 (SD = 0.0045%; CV = 1.90%), and 0.233% (SD = 0.0045%; CV = 1.94%) phytate-P, respectively. Dietary phytase was an average of 1,300 (SD = 102 FTU/kg; CV = 7.88%; Recovery = 86.7%), 1,271 (SD = 139 FTU/kg; CV = 10.96%; Recovery = 84.8%), and 1,299 FTU/kg (SD = 173 FTU/kg; CV = 13.32%; Recovery = 86.6%) for the starter, grower, and finisher diets, respectively. Prior to phytase analysis, feed samples were stored for an extended period at 4°C, which may have resulted in phytase concentrations being below anticipated levels (Singh, 2017).

Limestone Particle Size, Nutrient, and Solubility Analysis

The limestone particle size distribution is illustrated in **Figure 5.1**. In this experiment, coarse limestone had a d_{gw} of 910 µm with 99.57% of particles between 1,190 and 420 µm, whereas fine limestone d_{gw} was 200 µm with 100% of particles between 420 and 37 µm. Limestone nutrient and solubility analyses are shown in **Table 5.3**. As expected, analyzed nutrients between the 910 and 200 µm limestone particle size groups were numerically similar. However, solubility of the 200 µm limestone was numerically higher at 5 min (74.77 vs. 42.02%) and 15 min (95.36 vs. 78.36%) compared to the 900 µm. By 30 min, the difference in solubility between 910 and 200 µm limestone particle size was only 1.42% (96.89 vs. 98.31%). Although minimal, the 200 µm limestone still exhibited slightly higher solubility at this time point. In the present experiment, 2 distinct limestone particle size groups were created with minimal overlap in their particle size distribution. Based on the global survey by Gilani et al. (2022), the 200 µm limestone particle size group closely aligns with the US average. Targeting distinct particle sizes of limestone from the same origin was an attempt to differentiate a solubility effect or interaction with dietary calcium. Increasing calcium solubility can lead to higher concentrations of calcium-phytate complexes (Selle et al., 2009), which may interfere with nutrient utilization (Bedford and Rousseau, 2017). Research indicates that altering limestone particle size influences *in vitro* phytate-P hydrolysis (Manangi and Coon, 2007), *in vitro* solubility, and *in vivo* AID of calcium (Kim et al., 2019). In the present experiment, the d_{gw} range from 200 to 910 µm may not have provided a sufficiently broad particle size distribution to observe consistent effects between the 2 limestone particle size groups. Research by Kim et al. (2018) demonstrated that pulverized limestone (< 75 µm) has higher *in vitro* solubility and is more sensitive to calcium concentration and phytase activity than particulate limestone (402 µm). This also led to *in vivo* effects of particle size on gizzard pH and AID of calcium and P, despite the smaller d_{gw} difference between the 2 limestone particle sizes (327 µm) compared with the present experiment (710 µm).

Broiler Performance

Table 5.4 shows an independent orthogonal contrast between the performance of unchallenged and challenged birds when fed with similar dietary composition. Unchallenged birds had a higher BW (d 21, 26, and 35), higher FI (d 17 to 21, 1 to 21, 18 to 26, and 1 to 26), and lower FCR (d 1 to 21, 18 to 26, 1 to 26, and 1 to 35) compared to enterically challenged broilers ($P \leq 0.05$). However, mortality was not different between the unchallenged and challenged groups during the entire experiment ($P > 0.05$). In previous work, challenging broilers with *Eimeria* spp. (Wang et al., 2018), *C. perfringens* (Hussein et al., 2020), or both (Zanu et al., 2020a) led to growth impairment. Reduced growth in response to the enteric challenge was partly attributed to lower FI, potentially linked to immune system activation (Dantzer, 2004). The current enteric challenge model successfully reduced broiler performance without affecting mortality, aligning with certain characteristics of a subclinical NE challenge (Palliyeguru and Rose, 2014). However, as discussed in the companion paper (Tabish et al., 2024), there were limited intestinal lesions observed in the present experiment, which are essential criterion for diagnosing subclinical NE (Kaldhusdal and Hofshagen, 1992).

The influence of limestone particle size and calcium concentrations on broiler performance is shown in **Table 5.5**. Throughout the entire experiment, there were no significant main effects of limestone particle size on broiler performance or mortality ($P > 0.05$). As for calcium concentrations, broilers fed a diet with adequate and reduced calcium had a higher BW and FI compared to broilers fed low calcium throughout the entire experiment ($P \leq 0.05$). For all BW and FI measurements, the response to calcium concentration was quadratic ($P \leq 0.05$), except for FI from d 17 to 21, which exhibited a linear response ($P \leq 0.05$). Broilers fed diets with adequate calcium had a lower FCR compared to those fed diets with reduced and low calcium (1.133 vs. 1.147 and 1.176 g:g) from d 1 to 17 ($P \leq 0.05$; Quadratic: $P = 0.017$). From d 1 to 21 and d 1 to 26, FCR was lower for broilers fed diets with either adequate or reduced calcium compared to low calcium ($P \leq 0.05$; Linear: $P < 0.001$). A limestone particle size and calcium concentration interaction was observed for d 27 to 35 and 1 to 35 FCR ($P \leq 0.05$). Day 27 to 35 FCR for broilers in the 910 µm limestone particle size group exhibited a linear increase ($P < 0.001$) when calcium concentration decreased from adequate to low (**Figure 5.2 (a)**). In contrast, the 200 µm limestone particle size group showed a quadratic response ($P < 0.001$), with birds fed adequate and reduced calcium having similar FCR and low calcium leading to a higher FCR. A similar FCR response to calcium concentration in the 910 µm (Linear: $P < 0.001$) and 200 µm (Quadratic: $P = 0.007$) limestone particle size groups was observed from d 1 to 35 (**Figure 5.2 (b)**). As for mortality, broilers in the low calcium group had lower d 27 to 35 mortality compared to reduced calcium ($P \leq 0.05$; Quadratic: $P = 0.016$).

Under a mild enteric challenge with *Eimeria* spp. and *C. perfringens,* broiler performance was maintained using adequate and reduced calcium diets. However, decreasing dietary calcium 20-percentage points lowered FI, resulting in poor growth. Broilers have exhibited the ability to regulate calcium intake to meet physiological needs (Joshua and Mueller, 1979; Wilkinson et al., 2014), though this ability varies with disease state. Zanu et al. (2020a) observed reduced d 0 to 42 FI when broilers were fed high calcium diets under subclinical NE compared to no challenge. Under a clinical NE challenge, Paiva et al. (2013) reported similar BW gain, FI, and FCR for broilers fed diets with 0.60 or 0.90% calcium. Similarly, Walk et al. (2012a) observed that broilers not subjected to a disease exhibited comparable performance when fed diets containing 0.64 and 1.03% calcium. In this experiment, limestone particle size had no effect on BW or FI and limited effects on FCR. Previous research has shown inconsistent broiler performance responses to variations in limestone particle size (Manangi and Coon, 2007; Bradbury et al., 2018; Hu et al., 2020, Majeed et al., 2020). Interactions between calcium concentration and limestone particle size were observed for FCR from d 27 to 35 and 1 to 35. Diets with finer limestone combined with adequate calcium concentrations resulted in higher FCR compared to coarser limestone. This response may be attributed to the higher solubility of finer limestone. When combined with higher calcium concentrations, it could lower phytate-P hydrolysis (Manangi and Coon, 2007) and increase calcium-phytate complex formation (Selle et al., 2009). Consistent with the present findings, Majeed et al. (2020) and Lee et al. (2021) reported higher FCR when fine limestone was combined with low calcium compared to higher calcium concentrations. Overall, broiler performance was primarily influenced by calcium concentration.

Tibia Mineralization

Table 5.6 shows an independent orthogonal contrast between the tibia mineralization of unchallenged and challenged birds when fed with similar dietary composition. Tibia weight on d 21, as well as tibia shear strength and tibia ash on d 35, were similar between the unchallenged and enterically challenged broilers ($P > 0.05$). On d 21, tibia shear strength and ash content were higher in broilers from the unchallenged group compared to those challenged with *Eimeria* spp. and *C. perfringens* ($P \leq 0.05$). Similarly, tibia weight on d 35 was higher in the unchallenged group ($P \leq 0.05$). Tibia mineralization was reduced when assessed near the administration of the enteric challenge (d 21), which agrees with previous studies (Watson et al., 2005; Akbari Moghaddam Kakhki et al., 2019) and a recent meta-analysis (Shi et al., 2024). Enteric diseases have been associated with impaired bone resorption (Tompkins et al., 2023) and reduced nutrient digestibility (Amerah and Ravindran, 2015; Rochell et al., 2016), both of which contribute to decreased bone mineralization. Similarly, Oikeh et al. (2019) reported that coccidia-infected broilers had reduced plasma calcium and P concentrations, tibia breaking strength, and tibia ash. In the present experiment, tibia ash content was similar between unchallenged and challenged broilers on d 35. This finding is consistent with Zanu et al. (2021), who observed subclinical NE effects on d 16 tibia ash but not on d 29.

The effects of limestone particle size and calcium concentration on tibia mineralization are shown in **Table 5.7**. Limestone particle size did not affect tibia mineralization on d 21 and 35 ($P > 0.05$). Tibia weight on d 21 (Linear: $P < 0.001$) and d 35 (Quadratic: $P = 0.019$) was highest in broilers fed diets with adequate or reduced calcium compared to broilers fed diets with low calcium ($P \leq 0.05$). Similarly, broilers fed diets with adequate and reduced calcium had higher d 21 tibia shear strength (Linear: $P < 0.001$) and ash (Linear: $P < 0.001$), as well as d 35 tibia shear strength (Quadratic: $P = 0.003$) and ash (Linear: $P < 0.001$), compared to those fed diets with low calcium ($P \leq 0.05$). Furthermore, broilers in the adequate calcium group had a higher d 21 tibia shear strength and ash compared to reduced calcium ($P \leq 0.05$). No significant interactions between limestone particle size and calcium concentration were observed for tibia mineralization ($P > 0.05$). Under these enteric challenge conditions, reducing dietary calcium concentrations led to lower tibia mineralization, with tibia shear strength and tibia ash displaying similar responses to calcium concentrations. Regardless of a subclinical NE challenge, Zanu et al. (2020b) observed higher tibia breaking strength (d 16 and 29) and tibia ash (d 16 and 29) when dietary calcium was increased by 0.4 percentage units (e.g., 0.5 to 0.9% in the grower). Similarly, Paiva et al. (2014) reported increased tibia ash on d 35 with higher calcium concentrations (0.90 vs. 0.60%) during a clinical NE challenge. Enteric challenges may influence the tibia mineralization response to dietary calcium, as Walk et al. (2012b) found no effect of varying calcium concentrations (0.90, 0.75, 0.60, and 0.45%) on tibia ash in unchallenged broilers. Consistent with the present experiment, limestone particle size has been reported to have no effect on tibia ash (Manangi and Coon, 2007; Majeed et al., 2020). Variability in tibia ash assessment methods (Alkhtib et al., 2023) could explain why tibia ash values here exceed previously established ranges (Alkhtib et al., 2021), though similar values exceeding 50% have been reported (Walk et al., 2011; Paiva et al., 2014). Bone strength is influenced by multiple factors, including disease and nutrition (Rath et al., 2000). As observed in the present experiment, sufficient calcium concentrations are needed to maintain bone health under this mild enteric challenge using *Eimeria* spp. and *C. perfringens*. Relative to the performance results, reducing calcium by 10-percentage points still maintained tibia mineralization at 35 d of age.

Nutrient Digestibility

Table 5.8 shows an independent orthogonal contrast between the CP, fat, calcium, and P digestibility and AIDE of unchallenged and challenged birds when fed with similar dietary composition. Crude protein, calcium, and P digestibility on d 21 was higher when broilers were subjected to an enteric challenge ($P \leq 0.05$). However, unchallenged birds had a higher d 21 fat digestibility and AIDE compared to the challenged birds ($P \leq 0.05$). On d 35, calcium digestibility was higher in the challenged group compared to the unchallenged ($P \leq 0.05$). However, CP, fat, and P digestibility and AIDE did not differ between the unchallenged and challenged birds ($P > 0.05$). **Table 5.9** summarizes the independent orthogonal contrast for apparent essential and total ileal AA digestibility between the unchallenged and challenged groups. On d 21, the digestibility of all essential and total AA was higher in the enterically challenged broilers compared to the unchallenged group ($P \leq 0.05$). Unchallenged and challenged broilers had similar d 35 ileal digestibility for all essential and total AA ($P > 0.05$), except for Met digestibility. On d 35, broilers challenged with *Eimeria* spp. and *C. perfringens* had higher Met digestibility compared to the unchallenged broilers ($P \leq 0.05$). The independent orthogonal contrast for apparent non-essential AA digestibility is presented in **Table**

5.10. Broilers challenged with *Eimeria* spp. and *C. perfringens* had higher d 21 digestibility for all non-essential AA compared to the unchallenged broilers ($P \leq 0.05$). However, by d 35, the digestibility of all non-essential AA was similar between the challenged and unchallenged broilers ($P > 0.05$).

Nutrient digestibility of CP, calcium, P, and AA had an unexpected response when assessed after the co-challenge of *Eimeria* spp. and *C. perfringens*. However, fat digestibility and AIDE were reduced due to the enteric challenge, which is consistent with previous literature (Amerah and Ravindran, 2015; M'Sadeq et al., 2015). Impaired fat digestibility may result from interference with bile acid concentration caused by the enteric disease (Bansal et al., 2021). By the conclusion of the experiment, only calcium and Met digestibility remained higher in the challenged group. Digestible nutrient intake of CP, calcium, and P in challenged birds aligned with this response pattern of nutrient digestibility (**Appendix 4**, **Table S5.1**); however, unchallenged birds had higher total AA intake (**Appendix 4**, **Table S5.2**). It is understood that *Eimeria* spp. and *C. perfringens* can decrease nutrient digestibility (Williams, 2005; Guo et al., 2014; Rochell et al., 2016), as infections with these pathogens can induce intestinal damage (Wu et al., 2016; Kinstler et al., 2024). Although the response of CP, calcium, P, and AA digestibility is unexpected, Zanu et al. (2020c) also observed increased calcium and P digestibility when broilers were challenged with NE. Other factors could have influenced this response to *Eimeria* spp. and *C. perfringens* including the low virulence of the *C. perfringens* strain in the present experiment (Gharib-Naseri et al., 2019), altered cecal microbiota and short-chain fatty acid production (Stanley et al., 2012; Gharib-Naseri et al., 2019), or protease activity of *C. perfringens* (Blaschek and Solberg, 1978; Allison and Macfarlane, 1989).

Furthermore, the observed CP digestibility response in the present experiment was replicated in another experiment from our research group (Lin et al., 2024) that utilized the same enteric challenge model. Additional research is needed to understand the reasoning behind the increased CP, calcium, P, and AA digestibility when broilers were enterically challenged with this model.

The responses of CP, fat, calcium, and P digestibility and AIDE to varying limestone particle size and calcium concentrations on d 21 and 35 are shown in **Table 5.11**. Limestone particle size and calcium concentration did not influence CP or fat digestibility ($P > 0.05$). Broilers fed either adequate or reduced calcium diets had a lower d 35 calcium (Linear: $P = 0.001$) and P (Linear: $P < 0.001$) digestibility compared to broilers fed diets with low calcium ($P \leq 0.05$). On d 35, broilers fed diets with reduced calcium had an intermediate P digestibility. Broilers fed diets with adequate and reduced calcium had higher AIDE on d 35 compared to broilers fed low calcium diets ($P \leq 0.05$; Linear: $P < 0.001$). Limestone particle size and calcium concentration interactions were observed for d 21 calcium and P digestibility and AIDE ($P \leq 0.05$). On d 21, calcium digestibility in the 910 µm limestone particle size group showed a quadratic response ($P = 0.002$). Broilers fed an adequate calcium diet had the lowest calcium digestibility, while digestibility increased when broilers consumed a reduced or low calcium diet (**Figure 5.3 (a)**). In the 200 µm limestone particle size group, calcium digestibility exhibited a linear increase ($P < 0.001$) as calcium concentration decreased from adequate to low. Day 21 P digestibility linearly increased ($P < 0.001$) when calcium concentration decreased from adequate to low in the 910 µm limestone particle size group (**Figure 5.3 (b)**). However, a quadratic response ($P = 0.041$) to calcium concentration was observed in the 200 µm limestone particle size group. Broilers fed adequate and reduced calcium diets had similar P digestibility, whereas digestibility increased when broilers consumed a low calcium diet. Day 21 AIDE exhibited a quadratic response (*P* = 0.008) to calcium concentration in the 910 µm limestone particle size group (**Figure 5.3 (c)**). Broilers fed a diet with adequate calcium had lower AIDE compared to broilers fed reduced calcium. Broilers also had similar AIDE when either fed reduced or low calcium diets. In contrast, in the 200 µm limestone particle size group, d 21 AIDE was not different between varying calcium concentrations.

The influence of limestone particle size and calcium concentrations on apparent essential and total ileal AA digestibility is shown in **Table 5.12**. Limestone particle size and calcium concentration did not influence apparent essential and total ileal AA digestibility on d 21 or 35 ($P > 0.05$), except for Met (d 21), Met + Cys (d 21), and Thr (d 35) digestibility. Limestone particle size and calcium concentration interactions were observed for d 21 Met and Met + Cys digestibility ($P \leq 0.05$). Within the 910 µm limestone particle size group, Met digestibility linearly increased (*P* = 0.004) as calcium concentration decreased from adequate to low (**Figure 5.4 (a)**). Broilers fed an adequate calcium diet had lower d 21 Met digestibility compared to broilers fed a low calcium diet ($P \leq 0.05$). In contrast, calcium concentration in the 200 µm limestone particle size group did not influence d 21 Met digestibility. Day 21 Met + Cys digestibility also linearly increased (*P* = 0.009) in the 910 µm limestone particle size group as broilers had lower digestibility when fed an adequate calcium diet compared to a reduced calcium diet (**Figure 5.4 (b)**). Calcium concentration did not influence d 21 Met + Cys digestibility in the 200 µm limestone particle size group. Although the Tukey-Kramer mean separation was similar in the 200 µm limestone particle size group, d 21 Met + Cys digestibility exhibited a linear response to calcium concentration ($P = 0.010$). Although an interaction was observed for d 35 Thr digestibility ($P \leq 0.05$; **Figure 5.4 (c)**), the Tukey-Kramer multiple comparison test did not separate the means, and the 95% CLM overlapped across all treatments.

Limestone particle size and calcium concentration had minimal influence on apparent non-essential ileal AA digestibility (**Table 5.13**). Only d 21 (interaction) and d 35 (calcium concentration main effect) Cys digestibility was influenced by the dietary factors ($P \leq 0.05$). Day 21 Cys digestibility showed a quadratic increase ($P = 0.018$), with broilers fed a reduced calcium diet and 910 µm limestone particle size exhibiting higher digestibility compared to those fed adequate calcium and the same limestone particle size (**Figure 5.5**). In the 200 µm limestone particle size group, d 21 Cys digestibility linearly decreased ($P = 0.003$) with lower calcium concentrations. Broilers had higher Cys digestibility when fed an adequate calcium diet compared to a low calcium diet. Day 35 Cys digestibility was higher when broilers were fed adequate and reduced calcium diets compared to low calcium ($P \leq 0.05$; Quadratic: $P = 0.025$).

In this experiment, reducing dietary calcium concentration increased calcium and P digestibility. This suggests that higher dietary calcium concentrations likely increased calcium-phytate complex formation rather than exacerbating enteric disease severity, as CP, fat digestibility, and AIDE did not follow the same trend. In addition, higher calcium concentrations did not negatively affect performance, further supporting the hypothesis that increased calcium-phytate formation, rather than disease severity, influenced calcium and P digestibility. Lower calcium concentrations may reduce calcium-phytate interactions, assisting with phytase activity (Sebastian et al., 1996; Selle et al., 2009), which can improve nutrient digestibility. Also, birds fed lower dietary calcium may have exhibited an adaptive capacity to utilize these minerals by the conclusion of the study (Yan et al., 2005). In contrast to calcium and P digestibility, AIDE was lowered when decreasing dietary calcium by 20-percentage points. Adequate calcium concentrations used in this experiment may not have supported high insoluble calcium soap formation, which can influence nutrient utilization (Tancharoenrat and Ravindran, 2014). The effect of dietary calcium on energy utilization is variable, with differing responses reported (Walk et al., 2012b; Tancharoenrat and Ravindran, 2014; Akter et al., 2018). Other than calcium and P, the lack of consistent negative effects from higher dietary calcium concentrations on nutrient digestibility could indicate that the calcium concentrations used in this experiment did not exceed physiological need. Higher dietary calcium concentrations utilized in other studies have shown adverse effects on nutrient digestibility (Shafey and McDonald, 1991; Mutucumarana et al., 2014; Tancharoenrat and Ravindran, 2014). Nutrient digestibility was minimally affected by dietary calcium concentration and limestone particle size during this mild enteric challenge. However, interactions between calcium and limestone particle size were observed for calcium, P, Met, Met + Cys, Thr, Cys digestibility, and AIDE. As previously discussed, altering limestone particle size can affect its solubility, which can influence nutrient digestibility in response to dietary calcium. Calcium and P digestibility followed a similar response regardless of particles size, with the lowest digestibility at adequate calcium concentrations and the highest at the lowest calcium concentrations. Interestingly, AIDE and the digestibility of Met, Met + Cys, and Thr were all similar within the finest limestone particle size group, regardless of calcium concentration. This suggests that coarser limestone may increase variability in nutrient digestibility. Researchers have reported the effects of limestone particle size on nutrient digestibility (Bradbury et al., 2018; Kim et al., 2018; Kim et al., 2019; Majeed et al., 2020; Li et al., 2021). Majeed et al. (2020) reported that fine limestone particle size (190 µm) increased AA digestibility compared with coarse particle size (900 µm). These responses are likely related to limestone solubility, though they depend on additional dietary factors, including calcium and phytase concentrations (Bradbury et al., 2018; Kim et al., 2018; Kim et al., 2019; Majeed et al., 2020; Li et al., 2021).

In conclusion, the *Eimeria* spp. and *C. perfringens* challenge reduced BW and increased FCR, highlighting its negative impact on performance. Under this enteric challenge model, broiler performance, tibia mineralization, and nutrient digestibility were primarily influenced by dietary calcium concentrations. Reducing dietary calcium by 0.10 percentage units from recommended concentrations maintained performance and tibia mineralization, while a 0.20 percentage unit reduction impaired growth. Thus, lowering calcium beyond physiological needs impacted broiler performance under this mild enteric challenge. Limestone particle size had minimal effects in general, but did interact with calcium concentration to influence FCR and nutrient digestibility. Targeting optimal calcium concentrations and considering limestone particle size when formulating broiler diets may enhance broiler growth and nutrient digestibility under an enteric challenge with *Eimeria* spp. and *C. perfringens*. The severity and type of enteric disease may further modulate these interactions.

FUNDING SOURCE

This research was funded impart by a USDA-ARS agreement and by the Alabama Agricultural Experiment Station.

ACKNOWLEDGEMENTS

The authors are grateful for the graduate students, undergraduate students, and visiting research scholars from Drs. Pacheco, Rochell, Hauck, Dozier, Bailey, and Morey labs for their assistance during this experiment. Additionally, the authors want to express their gratitude to the Auburn University Charles. C. Miller Jr. Poultry Research Center and Poultry and Animal Nutrition Center staff for their constant support and contributions during each experiment. Lastly, the authors greatly appreciate Tucker Milling, LLC and AB Vista for providing all limestone used in this experiment and helping with dietary phytate-P and phytase activity analyses, respectively.

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Table 5.1. Ingredient composition (% as-fed) of the adequate, reduced, and low calcium (Ca) concentration diets[1] with varying limestone particle sizes (PS)[2] fed to YPM x Ross 708 male broilers from 1 to 35 d of age.

	Starter, 1 to 17 d			Grower, 18 to 26 d			Finisher, 27 to 35 d		
Ingredient	Adequate	Reduced	Low	Adequate	Reduced	Low	Adequate	Reduced	Low
Corn	55.46	55.46	55.46	56.82	56.82	56.82	60.42	60.42	60.42
Soybean meal, 48% CP	39.27	39.27	39.27	36.87	36.87	36.87	33.61	33.61	33.61
DL-methionine	0.37	0.37	0.37	0.36	0.36	0.36	0.33	0.33	0.33
Soy oil	1.83	1.83	1.83	2.84	2.84	2.84	2.95	2.95	2.95
L-lysine	0.21	0.21	0.21	0.18	0.18	0.18	0.14	0.14	0.14
L-threonine	0.13	0.13	0.13	0.11	0.11	0.11	0.08	0.08	0.08
L-valine	0.04	0.04	0.04	0.03	0.03	0.03	0.01	0.01	0.01
Dicalcium phosphate, 18% P	1.05	1.05	1.05	0.64	0.64	0.64	0.35	0.35	0.35
Limestone (calcium carbonate)	0.90	0.64	0.39	0.92	0.66	0.41	0.87	0.62	0.36
Inert filler[3] (sand)	0.00	0.26	0.51	0.00	0.26	0.51	0.00	0.26	0.51
Salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Trace mineral premix[4]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin premix[5]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Choline chloride	0.06	0.06	0.06	0.05	0.05	0.05	0.05	0.05	0.05
Phytase[6]	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Titanium dioxide (TiO_2)	–	–	–	0.50	0.50	0.50	0.50	0.50	0.50

[1] Calcium concentration was a two-step, 0.10 percentage unit reduction from the primary breeder's requirements for dietary Ca for each of the growth phases (Starter: Adequate (0.95 %), Reduced (0.85 %), Low (0.75 %); Grower: Adequate (0.85 %), Reduced (0.75 %), Low (0.65 %); Finisher: Adequate (0.75 %), Reduced (0.65 %), Low (0.55 %)).

[2] Limestone was ground using a 2-pair roller mill (Roskamp Champion Series 900-12, California Pellet Mill Co., Crawfordsville, IN) to achieve a PS of 910 µm (coarse) and 200 µm (fine).

[3] Sand was added at the expense of limestone to target reduced calcium concentrations while maintaining all other nutrients.

[4] Mineral premix included per kg of diet: Mn (manganese sulfate), 120 mg; Zn (zinc sulfate), 100 mg; Fe (iron sulfate monohydrate), 30 mg; Cu (tri-basic copper chloride), 8 mg; I (ethylenediamine dihydriodide), 1.4 mg; and Se (sodium selenite), 0.3 mg.

[5] Vitamin premix included per kg of diet: Vitamin A (Vitamin A acetate), 9,369 IU; Vitamin D (cholecalciferol), 3,307 IU; Vitamin E (DL-alpha tocopherol acetate), 33 IU; menadione (menadione sodium bisulfate complex), 2 mg; Vitamin B12 (cyanocobalamin), 0.02 mg; folacin (folic acid), 1.3 mg; D-pantothenic acid (calcium pantothenate), 15 mg; riboflavin (riboflavin), 11 mg; niacin (niacinamide), 44 mg; thiamine (thiamine mononitrate), 2.8 mg; D-biotin (biotin), 0.09 mg; and pyridoxine (pyridoxine hydrochloride), 3.9 mg.

[6] Quantum® Blue 5 G (AB Vista, Marlborough, Wiltshire, UK) provided 1,500 FTU/kg of phytase activity per kg of diet.

Table 5.2. Calculated and analyzed nutrient composition (% as-fed, unless otherwise noted) of the adequate, reduced, and low calcium (Ca) concentration diets[1] with varying limestone particle sizes (PS)[2] fed to YPM x Ross 708 male broilers from 1 to 35 d of age.

	Starter, 1 to 17 d			Grower, 18 to 26 d			Finisher, 27 to 35 d		
Calculated nutrients	Adequate	Reduced	Low	Adequate	Reduced	Low	Adequate	Reduced	Low
DM	88.61	88.61	88.61	88.73	88.73	88.73	88.69	88.69	88.69
AMEn, kcal/kg	2,975	2,975	2,975	3,050	3,050	3,050	3,100	3,100	3,100
CP	23.42	23.42	23.42	22.32	22.32	22.32	20.93	20.93	20.93
Ca	0.95	0.85	0.75	0.85	0.75	0.65	0.75	0.65	0.55
Non-phytate phosphorous	0.50	0.50	0.50	0.42	0.42	0.42	0.36	0.36	0.36
Digestible Lys	1.26	1.26	1.26	1.18	1.18	1.18	1.08	1.08	1.08
Digestible Met + Cys	0.95	0.95	0.95	0.92	0.92	0.92	0.86	0.86	0.86
Digestible Met	0.66	0.66	0.66	0.64	0.64	0.64	0.60	0.60	0.60
Digestible Thr	0.84	0.84	0.84	0.79	0.79	0.79	0.72	0.72	0.72
Digestible Val	0.96	0.96	0.96	0.91	0.91	0.91	0.84	0.84	0.84
Analyzed nutrients[3]									
DM[4]	87.99	87.91	87.70	87.71	87.86	87.71	88.22	88.18	88.31
Gross energy[5], kcal/kg	3,974	3,972	3,944	3,980	4,000	3,971	3,998	3,997	3,994
CP	24.75	22.97	22.02	22.93	22.88	22.27	21.21	21.56	21.04
Ca	0.72	0.63	0.64	0.59	0.52	0.45	0.60	0.46	0.38
Total phosphorus	0.59	0.59	0.59	0.52	0.50	0.49	0.46	0.44	0.45
Phytate-phosphorus[6]	0.25	0.25	0.26	0.24	0.24	0.24	0.23	0.23	0.24
Phytase activity[6], FTU/kg	1,217	1,365	1,360	1,283	1,375	1,150	1,283	1,405	1,215
Crude fat	4.72	4.76	4.67	5.54	5.61	5.47	5.55	5.41	5.46
Total Lys	–[7]	–	–	1.48	1.42	1.40	1.36	1.38	1.35
Total Met + Cys	–	–	–	1.06	1.03	1.03	0.96	1.02	1.00
Total Met	–	–	–	0.69	0.66	0.66	0.60	0.65	0.64
Total Thr	–	–	–	0.98	0.92	0.92	0.86	0.88	0.90
Total Val	–	–	–	1.16	1.12	1.10	1.05	1.09	1.05

[1] Calcium concentration was a two-step, 0.10 percentage unit reduction from the primary breeder's requirements for dietary Ca for each of the growth phases (Starter: Adequate (0.95 %), Reduced (0.85 %), Low (0.75 %); Grower: Adequate (0.85 %), Reduced (0.75 %), Low (0.65 %); Finisher: Adequate (0.75 %), Reduced (0.65 %), Low (0.55 %)).

[2] Limestone was ground using a 2-pair roller mill (Roskamp Champion Series 900-12, California Pellet Mill Co., Crawfordsville, IN) to achieve a PS of 910 µm (coarse) and 200 µm (fine).

[3] Crude protein, calcium, total phosphorus, crude fat, and AA profile analyses were performed by the University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO); provided are averages of analyzed nutrients for treatments with similar Ca concentrations.

[4] Dry matter content was determined by measuring moisture content (AOCS Am 5-04 method, filter bag (ANKOM); analyzed at the University of Missouri Agricultural Experiment Station Chemical Laboratories, Columbia, MO) and subtracting the moisture percentage from 100.

[5] Gross energy was analyzed at Auburn University (Auburn, AL); provided are averages of gross energy for treatments with similar Ca concentrations.

[6] Phytate-P and phytase activity were analyzed at an external laboratory (AB Vista, Plantation, FL); provided are average values for treatments with similar Ca concentrations.

[7] Amino acid content was only analyzed for grower and finisher diets to calculate apparent ileal digestibility.

Table 5.3. Nutrient and solubility analyses of the different limestone particle sizes (PS) fed to YPM x Ross 708 male broilers from 1 to 35 d of age.

	Limestone PS[1], µm	
Items	910	200
Nutrient analysis[2]		
Moisture, %	0.070	0.030
DM, %	99.93	99.97
Calcium, %	38.20	37.65
Phosphorus, %	0.010	<0.010
Magnesium, %	0.35	0.33
Potassium, %	0.010	0.020
Sodium, %	0.020	0.020
Iron, ppm	391.94	394.93
Manganese, ppm	28.92	29.00
Zinc, ppm	47.06	50.20
Copper, ppm	<0.010	<0.010
Solubility analysis[2,3], %		
5 min	42.02	74.77
15 min	78.36	95.36
30 min	96.89	98.31

[1] Limestone was ground using a 2-pair roller mill (Roskamp Champion Series 900-12, California Pellet Mill Co., Crawfordsville, IN) to achieve a PS of 910 µm (coarse) and 200 µm (fine).

[2] Limestone nutrient and solubility analyses were conducted by Chemuniqué (Pty) Ltd (Lanseria, South Africa).

[3] All limestone solubility results were performed in duplicate with the average of both runs reported.

Table 5.4. Independent orthogonal contrast for performance and mortality between unchallenged and challenged YPM x Ross 708 male broilers provided diets varying in limestone particle size (PS) and calcium (Ca) concentration from 1 to 35 d of age[1].

	200 µm limestone PS[2] and adequate Ca concentration[3]			
Measurements	Unchallenged	Challenged[4]	CLM[5]	*P*-value
BW, g/bird				
d 17	702	697	± 11	0.517
d 21	1,022	966	± 14	<0.001
d 26	1,567	1,478	± 27	<0.001
d 35	2,632	2,553	± 44	0.013
Feed intake, g/bird				
d 1 to 17	792	790	± 13	0.765
d 17 to 21	433	384	± 8	<0.001
d 1 to 21	1,225	1,180	± 16	<0.001
d 18 to 26	1,157	1,080	± 18	<0.001
d 1 to 26	1,949	1,861	± 28	<0.001
d 27 to 35	1,628	1,653	± 29	0.237
d 1 to 35	3,577	3,530	± 50	0.169
FCR, g:g				
d 1 to 17	1.123	1.131	± 0.008	0.163
d 1 to 21	1.195	1.213	± 0.011	0.023
d 18 to 26	1.345	1.398	± 0.020	<0.001
d 1 to 26	1.234	1.254	± 0.010	0.004
d 27 to 35	1.538	1.528	± 0.016	0.374
d 1 to 35	1.338	1.358	± 0.008	0.002
Mortality, %				
d 1 to 17	1.67	2.33	± 1.63	0.614
d 1 to 21	2.67	3.67	± 2.15	0.587
d 18 to 26	1.67	1.67	± 1.43	0.786
d 1 to 26	3.33	4.00	± 2.32	0.874
d 27 to 35	1.00	0.67	± 1.54	0.863
d 1 to 35	4.33	4.67	± 2.58	0.814

[1] Values are least square means of 10 replicate pens, with each pen containing 30 broilers at placement; Statistical significance was considered at $P \leq 0.05$.

[2] Limestone was ground using a 2-pair roller mill (Roskamp Champion Series 900-12, California Pellet Mill Co., Crawfordsville, IN) to achieve a PS of 910 µm (coarse) and 200 µm (fine).

[3] Calcium concentration was a two-step, 0.10 percentage unit reduction from the primary breeder's requirements for dietary Ca for each of the growth phases (Starter: Adequate (0.95 %); Grower: Adequate (0.85 %); Finisher: Adequate (0.75 %)).

[4] Broilers were enterically challenged with *Eimeria* spp. and *Clostridium perfringens*.

[5] CLM = 95% confidence limit for the mean.

Table 5.5. Performance and mortality of YPM x Ross 708 male broilers provided diets varying in limestone particle size (PS) and calcium (Ca) concentration from 1 to 35 d of age[1].

	Main effect									Interaction
	Limestone PS[2], µm (n = 30)				Ca concentration[3], (n = 20)					Limestone PS x Ca concentration
Measurements	910	200	CLM[4]	*P*-value	Adequate	Reduced	Low	CLM	*P*-value	*P*-value
BW, g/bird										
d 17	675	679	± 6	0.401	693^{a}	687^{a}	650^{b}	± 7	<0.001	0.320
d 21	942	948	± 7	0.259	964^{a}	956^{a}	914^{b}	± 9	<0.001	0.130
d 26	1,445	1,445	± 14	0.955	$1{,}479^{a}$	$1{,}460^{a}$	$1{,}397^{b}$	± 17	<0.001	0.988
d 35	2,515	2,508	± 24	0.603	$2{,}560^{a}$	$2{,}562^{a}$	$2{,}411^{b}$	± 28	<0.001	0.945
Feed intake, g/bird										
d 1 to 17	785	783	± 8	0.711	791^{a}	792^{a}	769^{b}	± 10	0.002	0.958
d 17 to 21	380	380	± 5	0.955	384^{a}	385^{a}	372^{b}	± 6	0.003	0.930
d 1 to 21	1,162	1,166	± 9	0.598	$1{,}179^{a}$	$1{,}175^{a}$	$1{,}139^{b}$	± 11	<0.001	0.499
d 18 to 26	1,065	1,068	± 11	0.718	$1{,}079^{a}$	$1{,}080^{a}$	$1{,}040^{b}$	± 13	<0.001	0.999
d 1 to 26	1,844	1,850	± 16	0.589	$1{,}862^{a}$	$1{,}869^{a}$	$1{,}808^{b}$	± 20	<0.001	0.804
d 27 to 35	1,651	1,630	± 16	0.074	$1{,}652^{a}$	$1{,}673^{a}$	$1{,}596^{b}$	± 20	<0.001	0.230
d 1 to 35	3,497	3,490	± 27	0.728	$3{,}533^{a}$	$3{,}555^{a}$	$3{,}393^{b}$	± 35	<0.001	0.974
FCR, g:g										
d 1 to 17	1.153	1.151	± 0.004	0.479	1.133^{c}	1.147^{b}	1.176^{a}	± 0.005	<0.001	0.127
d 1 to 21	1.230	1.225	± 0.006	0.239	1.214^{b}	1.223^{b}	1.246^{a}	± 0.008	<0.001	0.448
d 18 to 26	1.401	1.399	± 0.011	0.741	1.395	1.397	1.408	± 0.014	0.374	0.653
d 1 to 26	1.270	1.267	± 0.005	0.417	1.256^{b}	1.266^{b}	1.284^{a}	± 0.007	<0.001	0.928
d 27 to 35	1.538	1.534	± 0.009	0.473	1.511	1.520	1.576	± 0.012	<0.001	0.001
d 1 to 35	1.367	1.364	± 0.005	0.275	1.351	1.358	1.388	± 0.006	<0.001	0.001
Mortality, %										
d 1 to 17	1.33	2.44	± 0.95	0.139	1.83	1.83	2.00	± 1.17	0.992	0.887
d 1 to 21	2.33	3.33	± 1.24	0.275	3.17	2.50	2.83	± 1.52	0.671	0.882
d 18 to 26	1.67	1.33	± 0.83	0.386	1.83	1.17	1.50	± 1.01	0.711	0.144
d 1 to 26	3.00	3.78	± 1.35	0.362	3.67	3.00	3.50	± 1.66	0.760	0.580
d 27 to 35	1.78	1.22	± 0.90	0.365	1.17^{ab}	2.67^{a}	0.67^{b}	± 1.10	0.046	0.218
d 1 to 35	4.78	5.00	± 1.55	0.649	4.83	5.67	4.17	± 1.89	0.371	0.758

[a-c]Means within a row with different superscripts differ significantly ($P \leq 0.05$).

[1]Values are least square means of 30 (limestone PS main effect) and 20 (Ca concentration main effect) replicate pens, with each pen containing 30 broilers at placement.

[2]Limestone was ground using a 2-pair roller mill (Roskamp Champion Series 900-12, California Pellet Mill Co., Crawfordsville, IN) to achieve a PS of 910 µm (coarse) and 200 µm (fine).

[3]Calcium concentration was a two-step, 0.10 percentage unit reduction from the primary breeder's requirements for dietary Ca for each of the growth phases (Starter: Adequate (0.95 %), Reduced (0.85 %), Low (0.75 %); Grower: Adequate (0.85 %), Reduced (0.75 %), Low (0.65 %); Finisher: Adequate (0.75 %), Reduced (0.65 %), Low (0.55 %)).

[4]CLM = 95% confidence limit for the mean.

Table 5.6. Independent orthogonal contrast for tibia mineralization between unchallenged and challenged YPM x Ross 708 male broilers provided diets varying in limestone particle size (PS) and calcium (Ca) concentration on d 21 and 35[1].

	200 µm limestone PS[2] and adequate Ca concentration[3]			
Measurements	Unchallenged	Challenged[4]	CLM[5]	*P*-value
Tibia weight, g				
d 21	4.21	4.21	± 0.13	1.000
d 35	11.77	10.88	± 0.37	0.001
Shear strength, N				
d 21	363	314	± 20	0.001
d 35	490	478	± 22	0.427
Tibia ash, %				
d 21	54.26	53.68	± 0.39	0.040
d 35	52.98	53.16	± 0.59	0.668

[1] Values are least square means of 10 replicate pens, with each pen having left tibias collected from 4 broilers on d 21 and 35; Statistical significance was considered at $P \leq 0.05$.

[2] Limestone was ground using a 2-pair roller mill (Roskamp Champion Series 900-12, California Pellet Mill Co., Crawfordsville, IN) to achieve a PS of 910 µm (coarse) and 200 µm (fine).

[3] Calcium concentration was a two-step, 0.10 percentage unit reduction from the primary breeder's requirements for dietary Ca for each of the growth phases (Starter: Adequate (0.95 %), Reduced (0.85 %), Low (0.75 %); Grower: Adequate (0.85 %), Reduced (0.75 %), Low (0.65 %); Finisher: Adequate (0.75 %), Reduced (0.65 %), Low (0.55 %).

[4] Broilers were enterically challenged with *Eimeria* spp. and *Clostridium perfringens*.

[5] CLM = 95% confidence limit for the mean.

Table 5.7. Tibia mineralization of YPM x Ross 708 male broilers provided diets varying in limestone particle size (PS) and calcium (Ca) concentration on d 21 and 35[1].

	Main effect									Interaction
	Limestone PS[2], µm (n = 30)				Ca concentration[3], (n = 20)					Limestone PS x Ca concentration
Measurements	910	200	CLM[4]	*P*-value	Adequate	Reduced	Low	CLM	*P*-value	*P*-value
Tibia weight, g										
d 21	4.06	4.06	± 0.08	0.983	4.21^{a}	4.12^{a}	3.85^{b}	± 0.09	<0.001	0.522
d 35	10.81	10.73	± 0.26	0.634	11.15^{a}	11.07^{a}	10.09^{b}	± 0.32	<0.001	0.159
Shear strength, N										
d 21	291	282	± 12	0.285	316^{a}	287^{b}	256^{c}	± 15	<0.001	0.538
d 35	456	446	± 12	0.216	484^{a}	469^{a}	399^{b}	± 14	<0.001	0.956
Tibia ash, %										
d 21	52.93	53.19	± 0.26	0.132	53.64^{a}	53.05^{b}	52.49^{c}	± 0.32	<0.001	0.574
d 35	52.34	52.50	± 0.35	0.532	52.95^{a}	52.73^{a}	51.58^{b}	± 0.43	<0.001	0.532

[a-c] Means within a row with different superscripts differ significantly ($P \leq 0.05$).

[1] Values are least square means of 30 (limestone PS main effect) and 20 (Ca concentration main effect) replicate pens, with each pen having left tibias collected from 4 broilers on d 21 and 35.

[2] Limestone was ground using a 2-pair roller mill (Roskamp Champion Series 900-12, California Pellet Mill Co., Crawfordsville, IN) to achieve a PS of 910 µm (coarse) and 200 µm (fine).

[3] Calcium concentration was a two-step, 0.10 percentage unit reduction from the primary breeder's requirements for dietary Ca for each of the growth phases (Starter: Adequate (0.95 %), Reduced (0.85 %), Low (0.75 %); Grower: Adequate (0.85 %), Reduced (0.75 %), Low (0.65 %); Finisher: Adequate (0.75 %), Reduced (0.65 %), Low (0.55 %).

[4] CLM = 95% confidence limit for the mean.

Table 5.8. Independent orthogonal contrast for nutrient digestibility and apparent ileal digestible energy (AIDE) between unchallenged and challenged YPM x Ross 708 male broilers provided diets varying in limestone particle size (PS) and calcium (Ca) concentration on d 21 and 35[1].

	200 µm limestone PS[2] and adequate Ca concentration[3]			
Measurements	Unchallenged	Challenged[4]	CLM[5]	*P*-value
CP digestibility, %				
d 21	72.31	78.02	± 1.55	<0.001
d 35	77.60	77.91	± 2.41	0.870
Fat digestibility, %				
d 21	93.15	72.18	± 3.45	<0.001
d 35	92.16	90.90	± 4.56	0.898
Ca digestibility, %				
d 21	45.52	53.88	± 2.85	<0.001
d 35	43.75	60.28	± 5.78	<0.001
P[6] digestibility, %				
d 21	60.20	77.74	± 1.78	<0.001
d 35	68.24	73.63	± 4.03	0.088
AIDE[7], kcal/kg				
d 21	3,272	3,036	± 62	<0.001
d 35	3,314	3,332	± 46	0.556

[1] Values are least square means of 10 replicate pens, with each pen having ileal digesta collected from 8 and 6 broilers on d 21 and 35, respectively; Statistical significance was considered at $P \leq 0.05$.

[2] Limestone was ground using a 2-pair roller mill (Roskamp Champion Series 900-12, California Pellet Mill Co., Crawfordsville, IN) to achieve a PS of 910 µm (coarse) and 200 µm (fine).

[3] Calcium concentration was a two-step, 0.10 percentage unit reduction from the primary breeder's requirements for dietary Ca for each of the growth phases (Starter: Adequate (0.95 %), Reduced (0.85 %), Low (0.75 %); Grower: Adequate (0.85 %), Reduced (0.75 %), Low (0.65 %); Finisher: Adequate (0.75 %), Reduced (0.65 %), Low (0.55 %).

[4] Broilers were enterically challenged with *Eimeria* spp. and *Clostridium perfringens*.

[5] CLM = 95% confidence limit for the mean.

[6] P = Phosphorus.

[7] On a DM basis.

Table 5.9. Independent orthogonal contrast for apparent essential and total ileal amino acid (AA) digestibility between unchallenged and challenged YPM x Ross 708 male broilers provided diets varying in limestone particle size (PS) and calcium (Ca) concentration on d 21 and 35[1].

	200 µm limestone PS[2] and adequate Ca concentration[3]			
Digestibility, %	Unchallenged	Challenged[4]	CLM[5]	*P*-value
Met				
d 21	86.56	89.41	± 1.44	0.004
d 35	86.86	89.54	± 1.73	0.040
Met + Cys				
d 21	76.13	80.32	± 1.61	<0.001
d 35	78.88	80.35	± 2.37	0.371
Lys				
d 21	74.08	80.16	± 1.94	<0.001
d 35	80.20	79.09	± 3.18	0.574
Thr				
d 21	66.33	71.57	± 2.08	<0.001
d 35	72.40	71.46	± 3.23	0.631
Val				
d 21	71.67	76.08	± 2.10	0.003
d 35	76.56	75.51	± 2.81	0.585
Ile				
d 21	71.78	77.01	± 2.08	<0.001
d 35	78.14	76.71	± 2.76	0.428
Arg				
d 21	81.14	85.31	± 1.40	<0.001
d 35	85.72	84.81	± 2.19	0.511
Trp				
d 21	73.88	84.81	± 1.34	<0.001
d 35	80.41	78.28	± 2.87	0.256
Leu				
d 21	74.22	79.10	± 1.97	0.001
d 35	80.01	79.18	± 2.48	0.603
Phe				
d 21	75.37	79.99	± 1.70	<0.001
d 35	80.59	79.62	± 2.47	0.547
His				
d 21	76.48	79.28	± 1.69	0.018
d 35	81.12	79.69	± 2.23	0.331
Total AA				
d 21	74.04	78.73	± 1.71	<0.001
d 35	79.15	78.43	± 2.44	0.630

[1] Values are least square means of 10 replicate pens, with each pen having ileal digesta collected from 8 and 6 broilers on d 21 and 35, respectively; Statistical significance was considered at $P \leq 0.05$.

[2] Limestone was ground using a 2-pair roller mill (Roskamp Champion Series 900-12, California Pellet Mill Co., Crawfordsville, IN) to achieve a PS of 910 µm (coarse) and 200 µm (fine).

[3] Calcium concentration was a two-step, 0.10 percentage unit reduction from the primary breeder's requirements for dietary Ca for each of the growth phases (Starter: Adequate (0.95 %), Reduced (0.85 %), Low (0.75 %); Grower: Adequate (0.85 %), Reduced (0.75 %), Low (0.65 %); Finisher: Adequate (0.75 %), Reduced (0.65 %), Low (0.55 %).

[4] Broilers were enterically challenged with *Eimeria* spp. and *Clostridium perfringens*.

[5] CLM = 95% confidence limit for the mean.

Table 5.10. Independent orthogonal contrast for apparent non-essential ileal amino acid digestibility between unchallenged and challenged YPM x Ross 708 male broilers provided diets varying in limestone particle size (PS) and calcium (Ca) concentration on d 21 and 35[1].

	200 µm limestone PS[2] and adequate Ca concentration[3]			
Digestibility, %	Unchallenged	Challenged[4]	CLM[5]	*P*-value
Ala				
d 21	72.98	76.53	± 2.21	0.017
d 35	77.82	77.50	± 2.72	0.830
Asp				
d 21	71.56	76.20	± 1.66	<0.001
d 35	78.06	76.87	± 2.35	0.424
Cys				
d 21	57.20	63.64	± 2.20	<0.001
d 35	64.09	64.35	± 3.34	0.925
Glu				
d 21	79.59	83.78	± 1.24	<0.001
d 35	84.26	84.25	± 1.94	0.957
Gly				
d 21	67.33	72.08	± 2.07	0.001
d 35	73.75	71.74	± 2.92	0.286
Pro				
d 21	75.55	78.75	± 1.53	0.002
d 35	79.36	79.05	± 1.86	0.771
Ser				
d 21	71.72	76.81	± 1.62	<0.001
d 35	78.84	78.50	± 2.72	0.810
Tyr				
d 21	75.41	79.54	± 1.53	<0.001
d 35	79.60	80.21	± 2.44	0.719

[1] Values are least square means of 10 replicate pens, with each pen having ileal digesta collected from 8 and 6 broilers on d 21 and 35, respectively; Statistical significance was considered at $P \leq 0.05$.

[2] Limestone was ground using a 2-pair roller mill (Roskamp Champion Series 900-12, California Pellet Mill Co., Crawfordsville, IN) to achieve a PS of 910 µm (coarse) and 200 µm (fine).

[3] Calcium concentration was a two-step, 0.10 percentage unit reduction from the primary breeder's requirements for dietary Ca for each of the growth phases (Starter: Adequate (0.95 %), Reduced (0.85 %), Low (0.75 %); Grower: Adequate (0.85 %), Reduced (0.75 %), Low (0.65 %); Finisher: Adequate (0.75 %), Reduced (0.65 %), Low (0.55 %).

[4] Broilers were enterically challenged with *Eimeria* spp. and *Clostridium perfringens*.

[5] CLM = 95% confidence limit for the mean.

Table 5.11. Nutrient digestibility and apparent ileal digestible energy (AIDE) of YPM x Ross 708 male broilers provided diets varying in limestone particle size (PS) and calcium (Ca) concentration on d 21 and 35[1].

	Main effect									Interaction
	Limestone PS[2], µm (n = 30)				Ca concentration[3], (n = 20)					Limestone PS x Ca concentration
Measurements	910	200	CLM[4]	*P*-value	Adequate	Reduced	Low	CLM	*P*-value	*P*-value
CP digestibility, %										
d 21	77.79	77.31	± 0.86	0.426	77.34	77.58	77.73	± 1.07	0.862	0.096
d 35	78.26	78.66	± 1.27	0.699	78.15	79.14	78.08	± 1.61	0.522	0.471
Fat digestibility, %										
d 21	72.06	71.68	± 2.38	0.803	70.41	71.88	73.33	± 3.05	0.308	0.111
d 35	91.85	90.37	± 2.86	0.514	90.79	92.37	90.17	± 3.51	0.740	0.754
Ca digestibility, %										
d 21	61.76	62.22	± 1.66	0.714	52.08	63.93	69.95	± 2.07	<0.001	0.041
d 35	67.48	66.23	± 3.08	0.594	62.91[b]	65.31[b]	72.35[a]	± 3.85	0.002	0.353
P[5] digestibility, %										
d 21	80.60	80.75	± 0.99	0.720	77.76	80.36	83.90	± 1.25	<0.001	0.047
d 35	77.99	79.26	± 2.19	0.439	73.69[c]	78.78[b]	83.40[a]	± 2.70	<0.001	0.619
AIDE[6], kcal/kg										
d 21	3,024	3,023	± 31	0.979	2,987	3,054	3,030	± 40	0.052	0.010
d 35	3,339	3,314	± 24	0.137	3,361[a]	3,343[a]	3,277[b]	± 31	<0.001	0.408

[a-c]Means within a row with different superscripts differ significantly ($P \leq 0.05$).

[1] Values are least square means of 30 (limestone PS main effect) and 20 (Ca concentration main effect) replicate pens, with each pen having ileal digesta collected from 8 and 6 broilers on d 21 and 35, respectively.

[2] Limestone was ground using a 2-pair roller mill (Roskamp Champion Series 900-12, California Pellet Mill Co., Crawfordsville, IN) to achieve a PS of 910 µm (coarse) and 200 µm (fine).

[3] Calcium concentration was a two-step, 0.10 percentage unit reduction from the primary breeder's requirements for dietary Ca for each of the growth phases (Starter: Adequate (0.95 %), Reduced (0.85 %), Low (0.75 %); Grower: Adequate (0.85 %), Reduced (0.75 %), Low (0.65 %); Finisher: Adequate (0.75 %), Reduced (0.65 %), Low (0.55 %).

[4] CLM = 95% confidence limit for the mean.

[5] P = Phosphorus.

[6] On a DM basis.

Table 5.12. Apparent essential and total ileal amino acid (AA) digestibility of YPM x Ross 708 male broilers provided diets varying in limestone particle size (PS) and calcium (Ca) concentration on d 21 and 35[1].

	Limestone PS, µm (n = 30)				Ca concentration[2], (n = 20)					Limestone PS x Ca concentration
Digestibility, %	910	200	CLM[3]	*P*-value	Adequate	Reduced	Low	CLM	*P*-value	*P*-value
Met										
d 21	89.02	88.68	± 0.73	0.511	88.39	88.60	89.56	± 0.90	0.179	0.007
d 35	89.34	90.17	± 0.94	0.246	89.23	90.03	90.02	± 1.18	0.508	0.861
Met + Cys										
d 21	78.84	78.51	± 0.82	0.598	78.63	78.71	78.68	± 1.00	0.984	<0.001
d 35	79.38	80.25	± 1.24	0.339	80.15	80.77	78.53	± 1.57	0.084	0.883
Lys										
d 21	79.80	80.06	± 1.09	0.710	79.56	79.58	80.65	± 1.34	0.463	0.311
d 35	79.95	80.59	± 1.71	0.641	79.49	80.82	80.50	± 2.15	0.633	0.551
Thr										
d 21	72.26	71.48	± 1.06	0.308	72.22	71.19	72.20	± 1.29	0.483	0.167
d 35	71.99	73.00	± 1.65	0.389	71.89	72.81	72.79	± 2.11	0.742	0.043
Val										
d 21	76.24	75.81	± 1.07	0.584	75.67	75.82	76.59	± 1.31	0.603	0.350
d 35	76.63	77.23	± 1.51	0.606	76.29	77.76	76.75	± 1.91	0.490	0.300
Ile										
d 21	76.63	76.49	± 1.06	0.882	76.34	76.36	76.99	± 1.30	0.761	0.309
d 35	77.65	78.34	± 1.48	0.539	77.31	78.97	77.70	± 1.86	0.373	0.315
Arg										
d 21	85.25	85.34	± 0.75	0.846	85.03	85.23	85.63	± 0.92	0.676	0.703
d 35	85.87	86.18	± 1.16	0.771	85.31	86.74	86.02	± 1.47	0.358	0.370
Trp										
d 21	84.95	85.34	± 0.71	0.430	84.86	85.19	85.38	± 0.90	0.669	0.748
d 35	79.58	79.57	± 1.55	0.950	79.10	79.77	79.87	± 1.95	0.813	0.532
Leu										
d 21	79.11	78.88	± 1.00	0.771	78.45	78.85	79.68	± 1.23	0.385	0.297
d 35	79.47	80.05	± 1.34	0.582	79.42	80.54	79.32	± 1.69	0.467	0.538
Phe										
d 21	79.86	79.82	± 0.86	0.975	79.50	79.60	80.42	± 1.05	0.435	0.352
d 35	80.32	80.76	± 1.32	0.684	80.05	81.43	80.14	± 1.67	0.377	0.367
His										
d 21	78.89	79.01	± 0.91	0.834	78.78	78.99	79.07	± 1.12	0.924	0.461
d 35	80.33	80.68	± 1.19	0.726	80.25	81.44	79.82	± 1.51	0.233	0.297
Total AA										
d 21	78.51	78.50	± 0.87	0.999	78.18	78.38	78.95	± 1.06	0.604	0.342
d 35	79.00	79.47	± 1.30	0.646	78.83	80.10	78.76	± 1.64	0.371	0.370

[1] Values are least square means of 30 (limestone PS main effect) and 20 (Ca concentration main effect) replicate pens. [2] Calcium concentration was a two-step, 0.10 percentage unit reduction from the primary breeder's requirements for dietary Ca for each of the growth phases. [3] CLM = 95% confidence limit for the mean.

Table 5.13. Apparent non-essential ileal amino acid digestibility of YPM x Ross 708 male broilers provided diets varying in limestone particle size (PS) and calcium (Ca) concentration on d 21 and 35[1].

	Main effect									Interaction
	Limestone PS[2], µm (n = 30)				Ca concentration[3], (n = 20)					Limestone PS x Ca concentration
Digestibility, %	910	200	CLM[4]	*P*-value	Adequate	Reduced	Low	CLM	*P*-value	*P*-value
Ala										
d 21	76.62	76.47	± 1.12	0.869	75.76	76.42	77.45	± 1.37	0.240	0.286
d 35	78.08	78.38	± 1.44	0.806	77.96	78.86	77.87	± 1.82	0.650	0.573
Asp										
d 21	75.82	75.99	± 0.84	0.749	75.74	75.83	76.14	± 1.03	0.861	0.457
d 35	77.73	78.22	± 1.25	0.611	77.43	79.07	77.44	± 1.58	0.191	0.151
Cys										
d 21	60.30	60.95	± 1.19	0.419	60.89	61.20	59.79	± 1.50	0.339	<0.001
d 35	62.81	62.95	± 1.73	0.925	64.65^{a}	64.78^{a}	59.20^{b}	± 2.18	<0.001	0.705
Glu										
d 21	83.30	83.51	± 0.64	0.597	83.26	83.12	83.83	± 0.79	0.433	0.423
d 35	84.50	85.02	± 1.02	0.517	84.49	85.51	84.28	± 1.29	0.300	0.350
Gly										
d 21	71.55	71.64	± 1.04	0.889	71.28	71.69	71.81	± 1.28	0.828	0.215
d 35	72.74	73.02	± 1.55	0.837	72.49	73.89	72.27	± 1.96	0.389	0.342
Pro										
d 21	78.44	78.30	± 0.78	0.811	78.17	78.45	78.48	± 0.95	0.872	0.240
d 35	78.31	79.09	± 0.97	0.267	78.56	79.65	77.88	± 1.25	0.085	0.662
Ser										
d 21	76.07	76.53	± 0.92	0.458	75.74	76.50	76.66	± 1.16	0.446	0.123
d 35	77.95	78.06	± 1.37	0.968	78.59	78.96	76.46	± 1.76	0.056	0.399
Tyr										
d 21	80.71	80.41	± 0.84	0.626	79.67	80.69	81.32	± 1.06	0.084	0.995
d 35	79.35	80.02	± 1.39	0.517	79.88	80.33	78.84	± 1.71	0.447	0.528

[a,b] Means within a row with different superscripts differ significantly ($P \leq 0.05$).

[1] Values are least square means of 30 (limestone PS main effect) and 20 (Ca concentration main effect) replicate pens, with each pen having ileal digesta collected from 8 and 6 broilers on d 21 and 35, respectively.

[2] Limestone was ground using a 2-pair roller mill (Roskamp Champion Series 900-12, California Pellet Mill Co., Crawfordsville, IN) to achieve a PS of 910 µm (coarse) and 200 µm (fine).

[3] Calcium concentration was a two-step, 0.10 percentage unit reduction from the primary breeder's requirements for dietary Ca for each of the growth phases (Starter: Adequate (0.95 %), Reduced (0.85 %), Low (0.75 %); Grower: Adequate (0.85 %), Reduced (0.75 %), Low (0.65 %); Finisher: Adequate (0.75 %), Reduced (0.65 %), Low (0.55 %).

[4] CLM = 95% confidence limit for the mean.

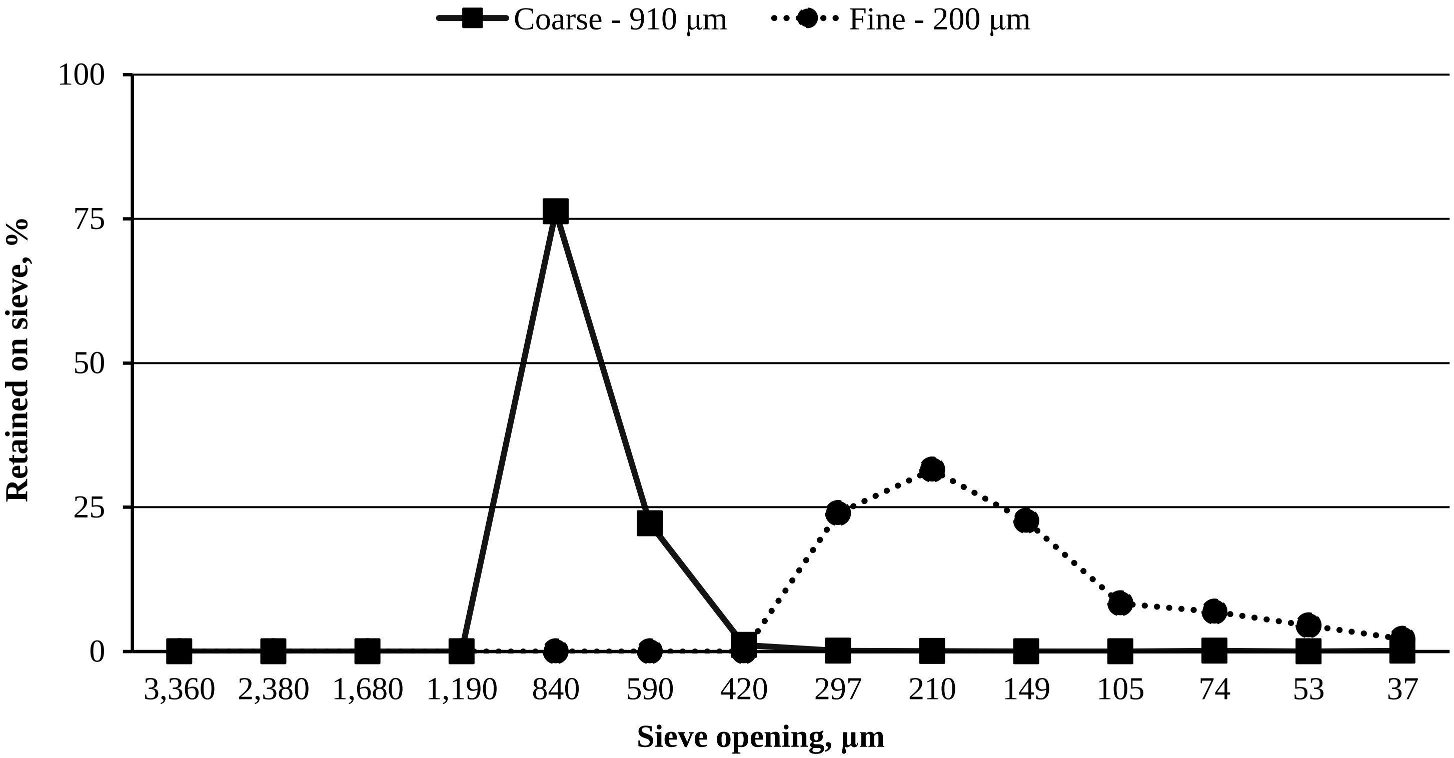
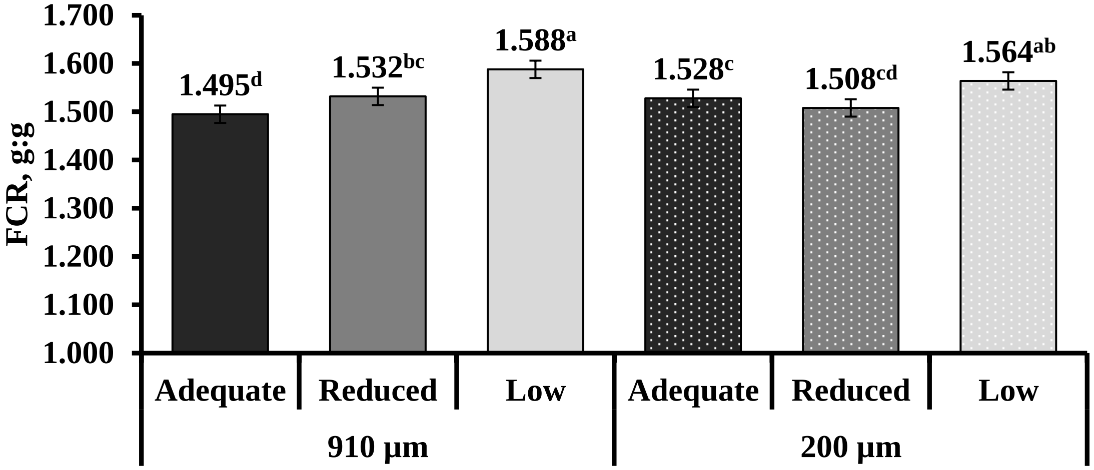


Figure 5.1. Coarse (910 µm) and fine (200 µm) limestone particle size distributions. Limestone was ground using a 2-pair roller mill (Roskamp Champion Series 900-12, California Pellet Mill Co., Crawfordsville, IN) to achieve distinct particle size distributions.

(a) Day 27 to 35 FCR



(b) Day 1 to 35 FCR

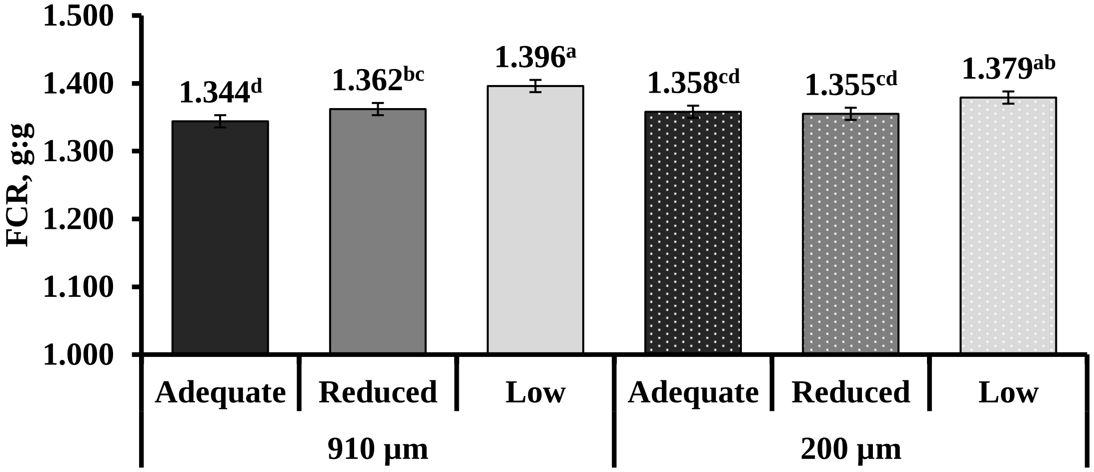
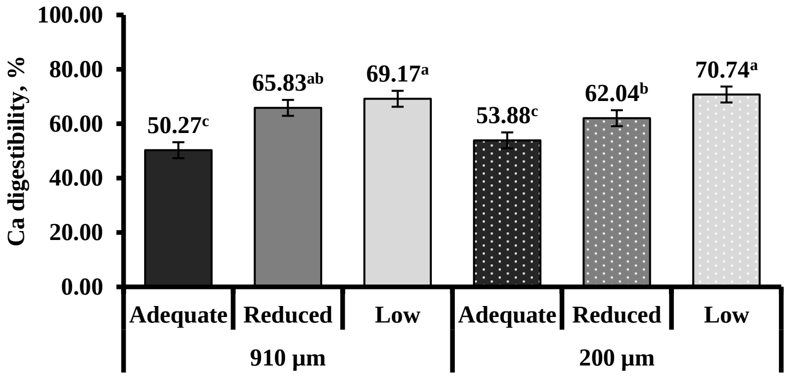


Figure 5.2. Interaction effects[1] of varying limestone particle size (910 and 200 µm) and Ca concentrations (adequate, reduced, and low) fed to YPM x Ross 708 male broilers on (**a**) d 27 to 35 (*P* = 0.001; CLM ± 0.018) and (**b**) d 1 to 35 FCR (*P* = 0.001; CLM ± 0.009). $^{a\text{-}d}$Means with different superscripts differ significantly ($P \leq 0.05$). [1]Interaction values are least square means of 10 replicate pens, with each pen containing 30 broilers at placement.

(a) Day 21 Calcium Digestibility



(b) Day 21 Phosphorus Digestibility

(c) Day 21 AIDE

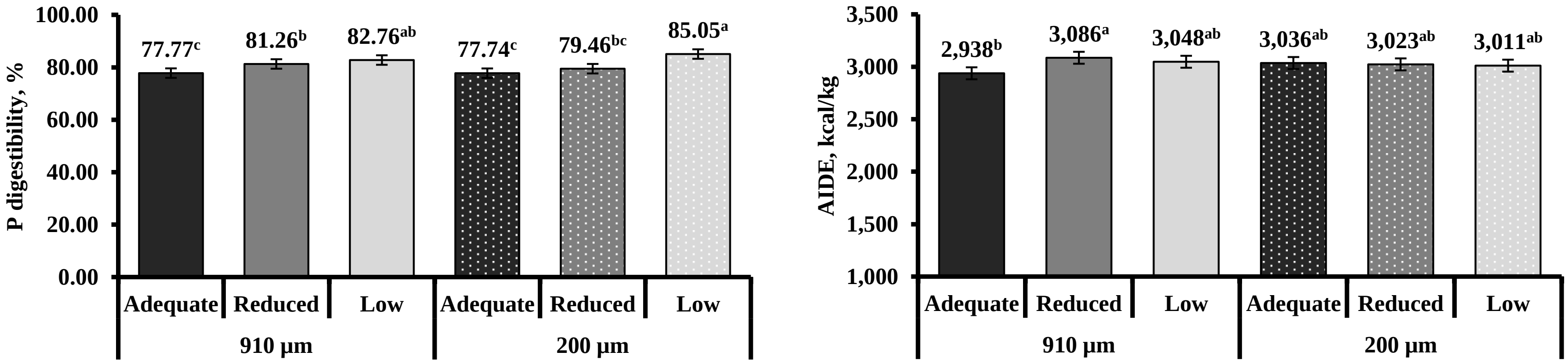
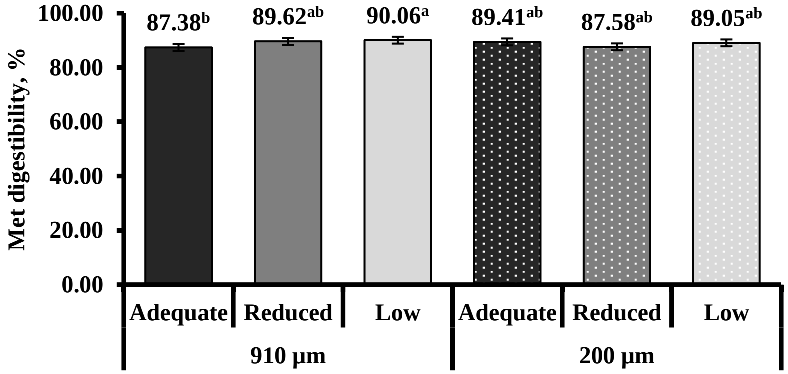
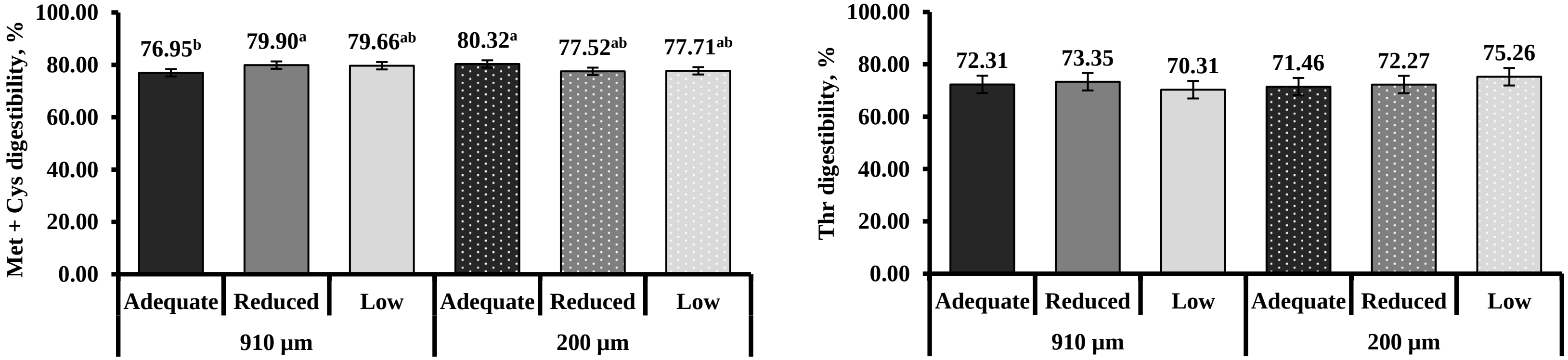


Figure 5.3. Interaction effects[1] of varying limestone particle size (910 and 200 µm) and Ca concentrations (adequate, reduced, and low) fed to YPM x Ross 708 male broilers on (**a**) d 21 Ca digestibility ($P = 0.041$; CLM ± 2.93), (**b**) d 21 P digestibility ($P = 0.047$; CLM ± 1.81), and (**c**) d 21 apparent ileal digestible energy (AIDE (on a DM basis); $P = 0.010$; CLM ± 57). $^{a\text{-}c}$Means with different superscripts differ significantly ($P \leq 0.05$). [1]Interaction values are least square means of 10 replicate pens, with each pen having ileal digesta collected from 8 broilers on d 21.

(a) Day 21 Met Digestibility




(b) Day 21 Met + Cys Digestibility

(c) Day 35 Thr Digestibility

Figure 5.4. Interaction effects[1] of varying limestone particle size (910 and 200 µm) and Ca concentrations (adequate, reduced, and low) fed to YPM x Ross 708 male broilers on (**a**) d 21 Met ($P = 0.007$; CLM ± 1.27), (**b**) d 21 Met + Cys digestibility ($P < 0.001$; CLM ± 1.41), and (**c**) d 35 Thr digestibility ($P = 0.043^{\dagger}$; CLM ± 3.34). ^{a,b}Means with different superscripts differ significantly ($P \leq 0.05$). [1]Interaction values are least square means of 10 replicate pens, with each pen having ileal digesta collected from 8 and 6 broilers on d 21 and 35, respectively. †Overall interaction was significantly different; however, Tukey-Kramer was unable to separate the means.

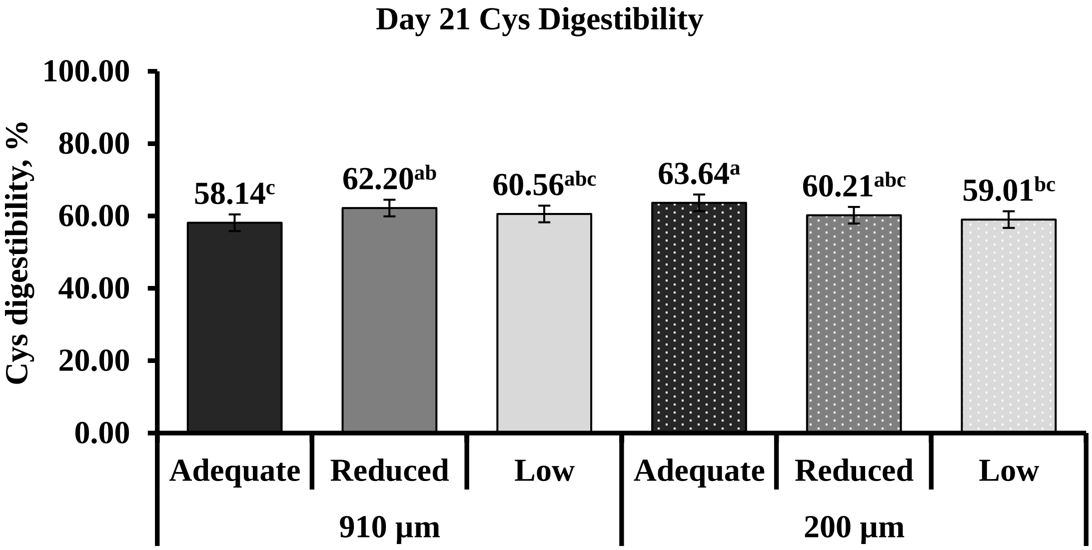


Figure 5.5. Interaction effects[1] of varying limestone particle size (910 and 200 µm) and Ca concentrations (adequate, reduced, and low) fed to YPM x Ross 708 male broilers on d 21 Cys digestibility ($P < 0.001$; CLM ± 2.30). $^{a-c}$Means with different superscripts differ significantly ($P \leq 0.05$). [1]Interaction values are least square means of 10 replicate pens, with each pen having ileal digesta collected from 8 broilers on d 21.

VI. CONCLUSIONS

The present dissertation research explored two nutritional strategies to address the removal of antibiotic growth promoters (**AGP**) in broiler feeding: (1) the use of feed additives such as phytase and calcium butyrate (**CB**), and (2) dietary manipulation, including adjustments in limestone particle size and dietary calcium concentration. Considering the extensive utilization of exogenous phytase and the search for effective AGP alternatives, understanding the interactions between phytase and microbiota-modulating feed additives (**MMFA**) is critical for optimizing broiler performance, nutrient utilization, and intestinal health. Additionally, the present research investigated how altering limestone particle size and calcium concentration influenced broiler performance and nutrient utilization during an enteric challenge. Collectively, these findings provide valuable insight into the complex interactions between feed additives and diet formulation, offering potential strategies to maintain broiler growth and health in the absence of AGP and under enteric challenge conditions.

The first study evaluated the effects of CB and bacitracin methylene disalicylate (**BMD**) combined with varying phytase concentrations on broiler performance, processing yields, nutrient digestibility (Part 1), as well as intestinal permeability, jejunum histomorphometry and tight-junction gene expression, and the diversity and metagenomic analysis of the cecal microbiome (Part 2). Bacitracin methylene disalicylate supplementation consistently improved broiler BW, feed conversion ratio (**FCR**), and crude protein digestibility compared to CB or no MMFA. However, these responses were dependent on dietary phytase concentrations. While intestinal permeability remained unaffected, interactions between phytase and MMFA influenced jejunal tight junction and mucin gene expression, as well as predicted bacterial metabolic function. Notably, MMFA effects were dependent on phytase concentration, emphasizing that findings from one phytase inclusion level may not be directly applicable to other phytase levels.

The second study evaluated the interactive effects of limestone particle size and calcium concentrations on broiler performance, tibia mineralization, and nutrient utilization during an enteric challenge with *Eimeria* spp. and *Clostridium perfringens*. In the context of this enteric challenge, dietary calcium levels were important for maintaining broiler performance, tibia mineralization, and nutrient utilization. A 0.10 percentage unit reduction in dietary calcium from recommended levels was sufficient to sustain growth and bone integrity, whereas a 0.20 percentage unit reduction impaired performance. While limestone particle size had minimal independent effects, it interacted with dietary calcium, influencing FCR and nutrient utilization. These findings reinforce the importance of optimizing dietary calcium levels and considering limestone particle size in diet formulation, particularly under enteric challenge conditions.

Collectively, these studies highlight the intricate relationships between phytase and MMFA, as well as between limestone particle size and calcium concentration in broiler nutrition. These findings suggest that dietary phytase inclusion influences the efficacy of MMFA, and that limestone particle size influences the responses to dietary calcium concentration during an enteric challenge. Given the widespread use of phytase, increasing emphasis on AGP alternatives, and the occurrence of elevated dietary calcium concentrations, understanding these interactions is paramount for developing feeding strategies that optimize broiler performance and health.

APPENDIX 1

SUPPLEMENTARY DATA ON CECAL MICROBIOME COMPOSITION, BETA DIVERSITY, AND CORRELATIONS WITH PERFORMANCE AND INTESTINAL HEALTH IN YPM X ROSS 708 MALE BROILERS

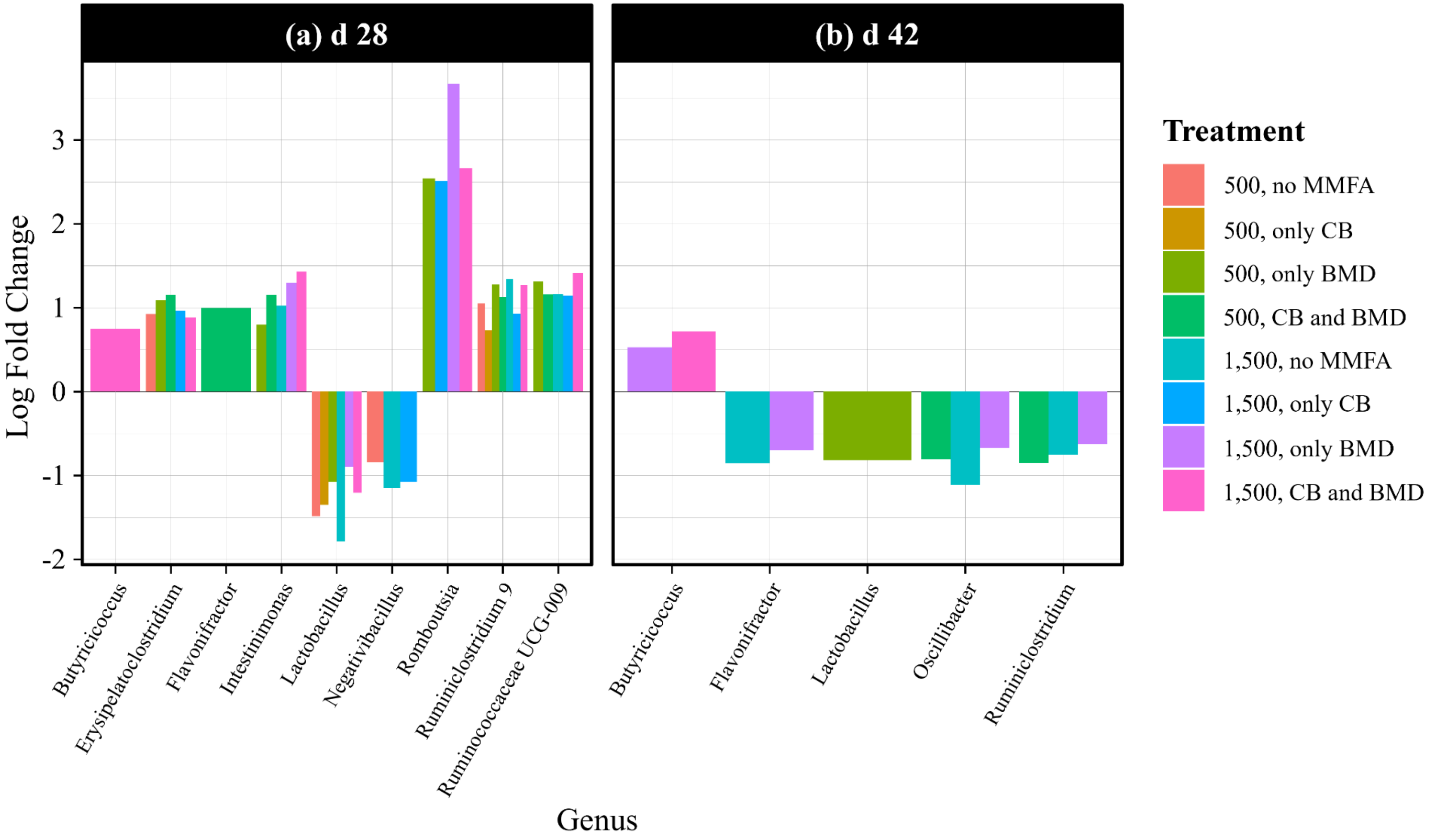


Figure S4.1. Differential abundance indicating log fold change in bacterial genera for (**a**) d 28 and (**b**) d 42 cecal microbiome of YPM x Ross 708 male broilers provided a negative control (NC) diet varying in phytase (500 or 1,500 FTU/kg) and microbiota modulating feed additive inclusion (MMFA; none, only calcium butyrate (CB), only bacitracin methylene disalicylate (BMD), or both CB and BMD). All log fold changes are significant ($P \leq 0.05$).

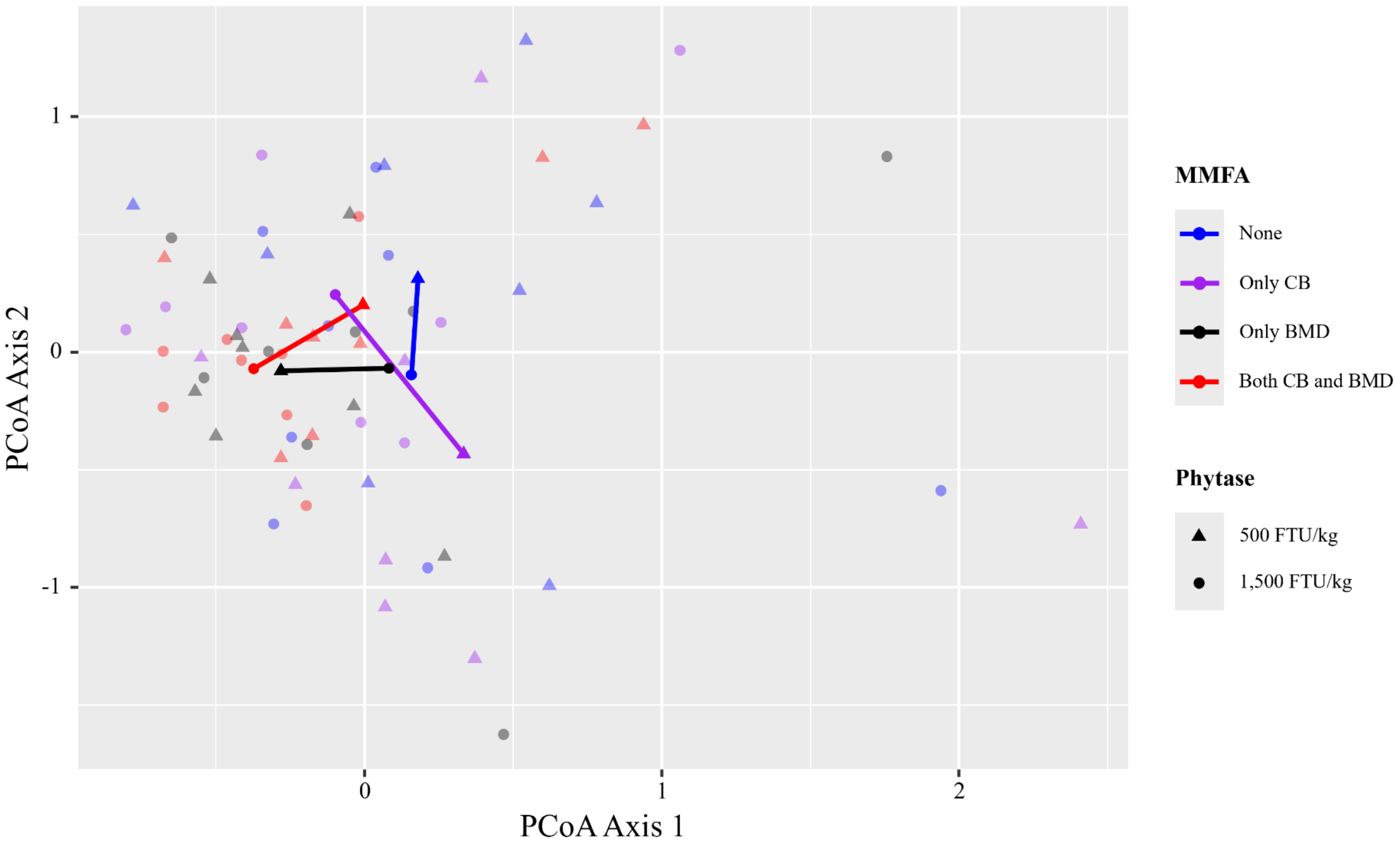


Figure S4.2. Beta diversity based on unweighted UniFrac distances for d 28 cecal microbiome of YPM x Ross 708 male broilers provided a negative control diet varying in phytase, calcium butyrate (CB), and bacitracin methylene disalicylate (BMD) inclusion. Interactions between phytase and microbiota modulating feed additives (MMFA) are plotted using mean centroids of the respective groups (*P* = 0.175).

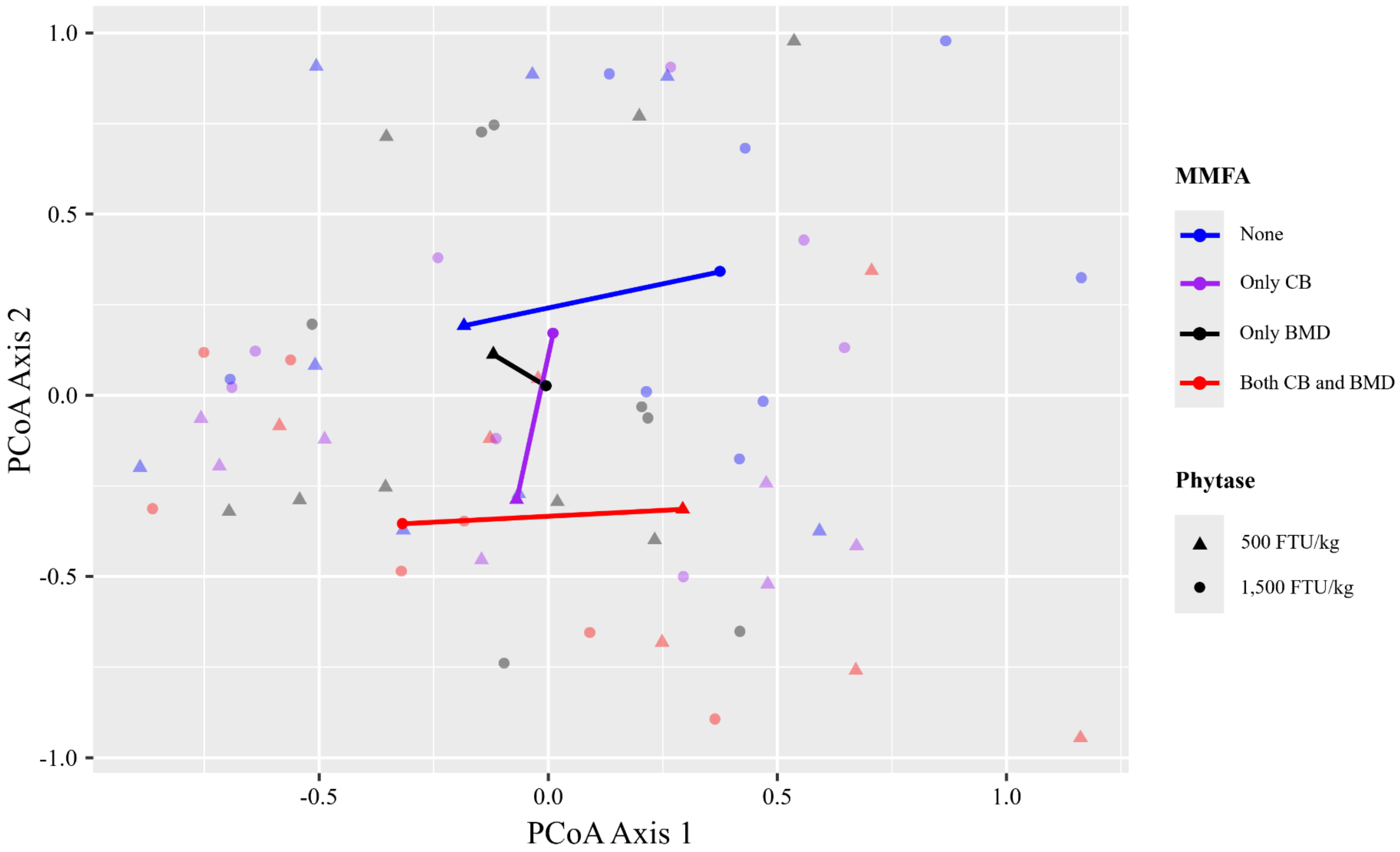


Figure S4.3. Beta diversity based on unweighted UniFrac distances for d 42 cecal microbiome of YPM x Ross 708 male broilers provided a negative control diet varying in phytase, calcium butyrate (CB), and bacitracin methylene disalicylate (BMD) inclusion. Interactions between phytase and microbiota modulating feed additives (MMFA) are plotted using mean centroids of the respective groups (*P* = 0.070).

Table S4.1. Pearson correlation coefficients (r) between d 28 live performance measurements and intestinal health and physiology measurements of YPM x Ross 708 male broilers provided a negative control diet varying in phytase, calcium butyrate (CB), and bacitracin methylene disalicylate (BMD) inclusion.

Measurement	BW	Feed conversion ratio	CP digestibility	AIDE[1]
Phytase, FTU/kg, main effect				
500				
Villus height	0.3630*	0.1350	0.1339	-0.0067
VH:CD ratio	0.5133**	-0.2663	0.1419	0.2029
Pielou's evenness	0.4449*	-0.5397**	0.2271	-0.1560
Observed features	-0.0770	-0.1492	-0.0158	-0.0801
1,500				
Villus height	0.0225	0.1919	0.0048	0.0023
VH:CD ratio	-0.0088	0.2192	0.3500	0.2626
Pielou's evenness	0.0661	0.0652	-0.0543	-0.0981
Observed features	0.4422*	-0.2941	0.1638	0.0473
MMFA[2], main effect				
None				
Villus height	0.2819	0.1739	0.1736	-0.1935
VH:CD ratio	-0.0093	0.2625	0.0076	0.1225
Pielou's evenness	-0.2449	0.4308	-0.4451	-0.5974*
Observed features	-0.0031	0.0839	-0.0570	-0.0182
Only CB				
Villus height	0.1708	0.4090	-0.1696	0.1272
VH:CD ratio	-0.3125	-0.1826	-0.0311	0.1808
Pielou's evenness	0.1855	-0.5816*	0.0649	0.1884
Observed features	0.0113	-0.5288	-0.1212	-0.0882
Only BMD				
Villus height	0.4201	-0.4589	0.0704	0.1747
VH:CD ratio	0.7012**	-0.1589	0.3189	0.4541
Pielou's evenness	0.0077	-0.3089	-0.0019	-0.4608
Observed features	-0.3743	-0.3255	-0.3979	-0.0027
Both CB and BMD				
Villus height	-0.1956	0.5398*	-0.2394	-0.2604
VH:CD ratio	-0.1602	0.4750	-0.0673	0.0267
Pielou's evenness	0.0739	0.1519	0.2555	0.0852
Observed features	0.1410	-0.0058	-0.0653	-0.0168

*$P \le 0.05$, **$P \le 0.01$, ***$P \le 0.001$; [1]AIDE = apparent ileal digestible energy.; [2]MMFA = microbiota modulating feed additives.

Table S4.2. Pearson correlation coefficients (r) between d 42 live performance measurements and intestinal health and physiology measurements of YPM x Ross 708 male broilers provided a negative control diet varying in phytase, calcium butyrate (CB), and bacitracin methylene disalicylate (BMD) inclusion.

Measurement	BW	Feed conversion ratio	CP digestibility	AIDE[1]
Phytase, FTU/kg, main effect				
500				
Villus height	-0.4234*	0.2672	0.4682**	0.1072
VH:CD ratio	-0.1489	0.2344	0.1300	0.0508
Pielou's evenness	0.1107	-0.0700	0.1218	0.1195
Observed features	-0.2596	0.1190	-0.0070	-0.0902
1,500				
Villus height	-0.1721	0.0424	0.2851	0.4017*
VH:CD ratio	-0.0245	0.0191	0.3022	0.3557*
Pielou's evenness	0.3511	-0.2892	-0.1949	-0.0565
Observed features	0.2123	0.0126	-0.2820	-0.1654
MMFA[2], main effect				
None				
Villus height	-0.0089	0.0472	0.5016	0.2850
VH:CD ratio	0.2231	0.3323	0.1557	0.1230
Pielou's evenness	0.4358	-0.3010	0.0563	-0.3553
Observed features	0.4207	-0.3879	-0.2993	-0.5348*
Only CB				
Villus height	0.2146	-0.0644	0.2286	0.2603
VH:CD ratio	0.0808	-0.1122	-0.1977	0.4034
Pielou's evenness	0.5216	-0.6112*	0.2693	-0.1042
Observed features	-0.0406	0.0927	0.2299	0.2348
Only BMD				
Villus height	-0.4719	0.4483	0.5167*	0.1840
VH:CD ratio	-0.4054	0.3059	0.7139**	0.2624
Pielou's evenness	0.2049	0.1313	-0.2212	0.0995
Observed features	-0.3182	0.4123	-0.1426	0.0247
Both CB and BMD				
Villus height	0.0513	0.0621	0.1525	0.1956
VH:CD ratio	0.1802	-0.1416	0.1064	-0.0445
Pielou's evenness	-0.2057	-0.2506	-0.0593	0.5879*
Observed features	0.0585	-0.1281	-0.3764	0.0719

*$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$; [1]AIDE = apparent ileal digestible energy.; [2]MMFA = microbiota modulating feed additives.

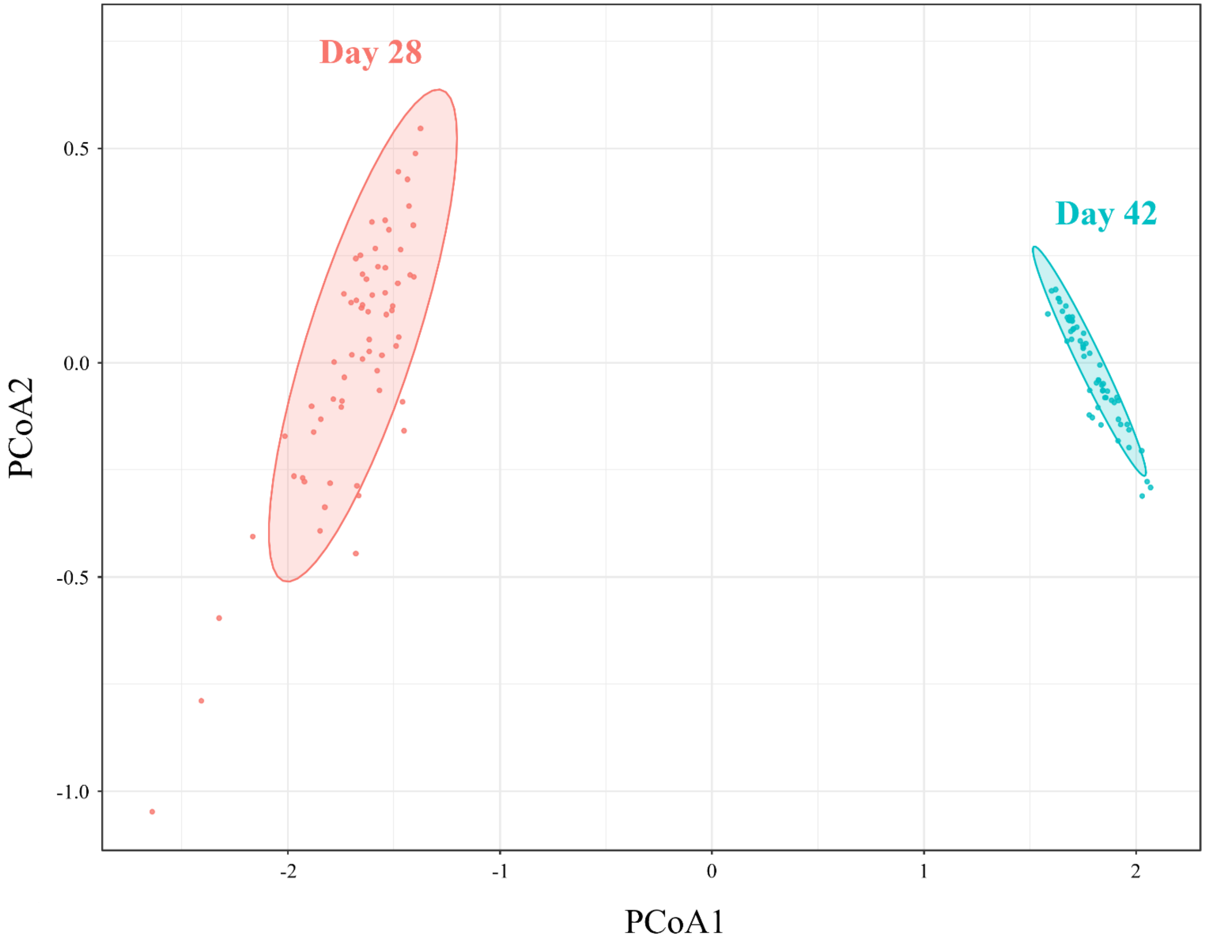


Figure S4.4. Beta diversity based on unweighted UniFrac distances for d 28 and 42 cecal microbiome of YPM x Ross 708 male broilers provided a negative control diet varying in phytase, calcium butyrate, and bacitracin methylene disalicylate inclusion. Beta diversity between sampling days was significant (*P* = 0.001).

APPENDIX 2

NUMBER OF UP- AND DOWN-REGULATED DIFFERENTIALLY EXPRESSED GENES BASED ON PREDICTED METAGENOME OF THE CECAL MICROBIOTA IN YPM X ROSS 708 MALE BROILERS

Number of up- and down-regulated differentially expressed genes (DEGs) based on predicted metagenome of the cecal microbiota compared between YPM x Ross 708 male broilers provided a negative control (NC; 0 FTU/kg) diet and a NC with phytase supplementation (500 or 1,500 FTU/kg) on d 28 and 42.

DEGs d 28: NC + 500 FTU/kg vs. NC (103 DEGs)						
GeneID	**baseMean**	**log2FoldChange**	**lfcSE**	**stat**	**pvalue**	**padj**
K00692	29.06869	-4.16777	1.19741	-3.48064	5.0021E-04	1.5847E-02
K00897	29.06869	-4.16777	1.19741	-3.48064	5.0021E-04	1.5847E-02
K12268	29.06869	-4.16777	1.19741	-3.48064	5.0021E-04	1.5847E-02
K12269	29.06869	-4.16777	1.19741	-3.48064	5.0021E-04	1.5847E-02
K12270	29.06869	-4.16777	1.19741	-3.48064	5.0021E-04	1.5847E-02
K01263	621.63995	-2.49140	0.54537	-4.56823	4.9185E-06	5.3759E-04
K10353	621.63995	-2.49140	0.54537	-4.56823	4.9185E-06	5.3759E-04
K13714	588.82764	-2.42254	0.56434	-4.29265	1.7655E-05	1.2384E-03
K07006	664.92748	-2.32379	0.49906	-4.65635	3.2187E-06	4.3975E-04
K08659	2752.36827	-2.31825	0.41983	-5.52183	3.3548E-08	3.6285E-05
K02077	750.88996	-2.18727	0.47653	-4.59004	4.4315E-06	5.0986E-04
K02248	710.67802	-2.17801	0.46254	-4.70882	2.4915E-06	4.0344E-04
K13531	704.63115	-2.17212	0.46208	-4.70076	2.5920E-06	4.0472E-04
K02246	706.93440	-2.16737	0.46354	-4.67567	2.9299E-06	4.1988E-04
K08728	721.78538	-2.13324	0.48866	-4.36550	1.2684E-05	9.6180E-04
K02074	721.82127	-2.13280	0.48870	-4.36419	1.2759E-05	9.6180E-04
K02075	721.82127	-2.13280	0.48870	-4.36419	1.2759E-05	9.6180E-04
K06994	702.15337	-2.12431	0.50305	-4.22283	2.4125E-05	1.4650E-03
K03697	1517.34752	-2.08919	0.39627	-5.27207	1.3489E-07	5.8975E-05
K05910	690.17820	-2.07959	0.50313	-4.13332	3.5755E-05	1.9375E-03
K09384	801.66672	-2.04795	0.44250	-4.62815	3.6894E-06	4.7442E-04
K02859	748.62318	-2.03637	0.47564	-4.28133	1.8578E-05	1.2476E-03
K19005	1530.30557	-2.01737	0.41235	-4.89235	9.9640E-07	2.2455E-04
K18892	779.37913	-2.00292	0.44785	-4.47234	7.7367E-06	7.7256E-04
K00869	781.68238	-1.99917	0.44898	-4.45265	8.4819E-06	7.7256E-04
K00938	781.68238	-1.99917	0.44898	-4.45265	8.4819E-06	7.7256E-04
K01597	781.68238	-1.99917	0.44898	-4.45265	8.4819E-06	7.7256E-04
K01641	781.68238	-1.99917	0.44898	-4.45265	8.4819E-06	7.7256E-04
K06198	781.68238	-1.99917	0.44898	-4.45265	8.4819E-06	7.7256E-04
K18104	713.58755	-1.98959	0.45840	-4.34033	1.4227E-05	1.0542E-03
K13678	750.49619	-1.98593	0.46327	-4.28678	1.8128E-05	1.2384E-03
K14188	796.26932	-1.92568	0.45073	-4.27232	1.9345E-05	1.2476E-03
K03739	796.36422	-1.92472	0.45058	-4.27163	1.9405E-05	1.2476E-03
K10041	805.98171	-1.91844	0.43676	-4.39245	1.1208E-05	9.5975E-04
K02244	808.52019	-1.91504	0.43731	-4.37917	1.1913E-05	9.5975E-04

DEGs d 28: NC + 500 FTU/kg vs. NC (103 DEGs)

GeneID	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj
K03095	808.52019	-1.91504	0.43731	-4.37917	1.1913E-05	9.5975E-04
K07177	808.52019	-1.91504	0.43731	-4.37917	1.1913E-05	9.5975E-04
K08987	808.52019	-1.91504	0.43731	-4.37917	1.1913E-05	9.5975E-04
K16169	808.28496	-1.91504	0.43760	-4.37625	1.2074E-05	9.5975E-04
K03367	800.56236	-1.91401	0.45040	-4.24960	2.1415E-05	1.3202E-03
K10040	1615.96032	-1.91372	0.39638	-4.82793	1.3796E-06	2.6225E-04
K01071	817.52333	-1.89448	0.42941	-4.41185	1.0249E-05	9.1446E-04
K05823	840.76101	-1.85592	0.49927	-3.71729	2.0137E-04	8.1760E-03
K03740	819.46658	-1.83514	0.45083	-4.07058	4.6897E-05	2.3841E-03
K18891	876.98607	-1.78785	0.45015	-3.97168	7.1366E-05	3.4668E-03
K02236	869.38302	-1.76470	0.41529	-4.24934	2.1440E-05	1.3202E-03
K02242	859.21176	-1.75292	0.41911	-4.18252	2.8830E-05	1.6585E-03
K03293	2953.80941	-1.74948	0.35328	-4.95205	7.3436E-07	2.0066E-04
K07707	1850.57844	-1.73553	0.38541	-4.50310	6.6970E-06	7.1413E-04
K06286	882.83166	-1.72821	0.40258	-4.29286	1.7639E-05	1.2384E-03
K01101	883.86239	-1.72821	0.40310	-4.28726	1.8089E-05	1.2384E-03
K00158	903.82824	-1.72083	0.44244	-3.88943	1.0048E-04	4.6734E-03
K08161	2096.10253	-1.69390	0.40283	-4.20503	2.6105E-05	1.5423E-03
K12555	932.96494	-1.65551	0.44032	-3.75975	1.7009E-04	7.0821E-03
K09155	2032.23130	-1.62285	0.37984	-4.27242	1.9337E-05	1.2476E-03
K12700	1244.07169	-1.61657	0.51129	-3.16172	1.5684E-03	3.6281E-02
K05964	1270.18218	-1.51074	0.48415	-3.12039	1.8061E-03	4.0704E-02
K05966	1270.32913	-1.51065	0.48412	-3.12038	1.8062E-03	4.0704E-02
K01256	1352.01923	-1.51033	0.47521	-3.17822	1.4818E-03	3.4831E-02
K03975	1366.87037	-1.49949	0.46957	-3.19331	1.4065E-03	3.4163E-02
K16209	959.22751	-1.47093	0.41951	-3.50634	4.5432E-04	1.5279E-02
K03319	1320.31813	-1.46964	0.48286	-3.04362	2.3375E-03	4.8234E-02
K00867	1411.75271	-1.46308	0.44787	-3.26675	1.0879E-03	2.9180E-02
K03316	1418.42429	-1.45728	0.44767	-3.25526	1.1329E-03	3.0201E-02
K07305	1423.32739	-1.43113	0.44688	-3.20253	1.3623E-03	3.3430E-02
K00364	1428.37351	-1.42212	0.43139	-3.29659	9.7867E-04	2.8336E-02
K13256	1434.61167	-1.41959	0.43346	-3.27500	1.0566E-03	2.8693E-02
K16012	1434.88807	-1.41959	0.43342	-3.27532	1.0554E-03	2.8693E-02
K16013	1434.88807	-1.41959	0.43342	-3.27532	1.0554E-03	2.8693E-02
K00851	1404.69798	-1.41472	0.44194	-3.20117	1.3687E-03	3.3430E-02
K03549	1605.19750	-1.40741	0.40328	-3.48988	4.8324E-04	1.5847E-02
K01823	1458.54432	-1.39732	0.43155	-3.23794	1.2040E-03	3.0948E-02
K00135	1448.61210	-1.39613	0.43346	-3.22087	1.2780E-03	3.1747E-02
K09963	1789.09990	-1.39262	0.29423	-4.73306	2.2116E-06	3.8676E-04

DEGs d 28: NC + 500 FTU/kg vs. NC (103 DEGs)

GeneID	**baseMean**	**log2FoldChange**	**lfcSE**	**stat**	**pvalue**	**padj**
K03483	1253.26001	-1.32687	0.43023	-3.08411	2.0416E-03	4.3921E-02
K03492	1824.66206	-1.31877	0.40799	-3.23236	1.2277E-03	3.0948E-02
K02798	1474.10452	-1.31570	0.43309	-3.03790	2.3823E-03	4.8670E-02
K10039	1226.14476	-1.29148	0.35617	-3.62605	2.8779E-04	1.1135E-02
K01007	1657.76439	-1.27201	0.37911	-3.35528	7.9286E-04	2.3742E-02
K01259	2090.61459	-1.25869	0.36690	-3.43063	6.0219E-04	1.8283E-02
K02245	1433.76026	-1.23414	0.39892	-3.09372	1.9766E-03	4.3209E-02
K02781	1718.49819	-1.21886	0.39247	-3.10564	1.8986E-03	4.2211E-02
K02761	8953.04755	-1.20913	0.22736	-5.31816	1.0482E-07	5.0920E-05
K01910	1687.94157	-1.12890	0.34935	-3.23144	1.2317E-03	3.0948E-02
K01916	3398.87672	-1.06432	0.27978	-3.80406	1.4235E-04	6.1727E-03
K02440	2172.91485	-1.04558	0.32276	-3.23949	1.1974E-03	3.0948E-02
K01215	1338.00614	-1.02960	0.33158	-3.10512	1.9020E-03	4.2211E-02
K01615	4213.64861	1.01046	0.18045	5.59974	2.1468E-08	3.1286E-05
K11691	2691.30633	1.03769	0.21278	4.87673	1.0786E-06	2.2455E-04
K11614	2769.29007	1.05970	0.20638	5.13469	2.8261E-07	1.0296E-04
K13677	1750.28927	1.14874	0.27660	4.15303	3.2810E-05	1.8158E-03
K05524	1139.33144	1.15865	0.37901	3.05705	2.2353E-03	4.6536E-02
K11528	583.89680	1.19402	0.33300	3.58561	3.3629E-04	1.2355E-02
K07654	584.02450	1.19413	0.33294	3.58664	3.3496E-04	1.2355E-02
K03430	817.86131	1.27943	0.39386	3.24840	1.1606E-03	3.0495E-02
K06386	439.71385	1.44751	0.47905	3.02163	2.5142E-03	4.9964E-02
K01174	388.31532	1.58042	0.50106	3.15412	1.6098E-03	3.6849E-02
K00355	359.94090	1.85610	0.57157	3.24735	1.1648E-03	3.0495E-02
K06426	333.10309	1.92946	0.56897	3.39116	6.9598E-04	2.0985E-02
K02456	333.42187	2.00700	0.53463	3.75399	1.7404E-04	7.1784E-03
K18940	306.35357	2.01391	0.57332	3.51273	4.4354E-04	1.5065E-02
K00019	88.43631	2.27812	0.70455	3.23344	1.2231E-03	3.0948E-02
K13530	88.43631	2.27812	0.70455	3.23344	1.2231E-03	3.0948E-02

Number of up- and down-regulated differentially expressed genes (DEGs) based on predicted metagenome of the cecal microbiota compared between YPM x Ross 708 male broilers provided a negative control (NC; 0 FTU/kg) diet and a NC with phytase supplementation (500 or 1,500 FTU/kg) on d 28 and 42.

DEGs d 28: NC + 1,500 FTU/kg vs. NC (184 DEGs)						
GeneID	**baseMean**	**log2FoldChange**	**lfcSE**	**stat**	**pvalue**	**padj**
K00692	29.06869	-5.29606	1.22185	-4.33447	1.4611E-05	6.5708E-04
K00897	29.06869	-5.29606	1.22185	-4.33447	1.4611E-05	6.5708E-04
K12268	29.06869	-5.29606	1.22185	-4.33447	1.4611E-05	6.5708E-04
K12269	29.06869	-5.29606	1.22185	-4.33447	1.4611E-05	6.5708E-04
K12270	29.06869	-5.29606	1.22185	-4.33447	1.4611E-05	6.5708E-04
K01263	621.63995	-3.18910	0.54583	-5.84270	5.1361E-09	1.3008E-06
K10353	621.63995	-3.18910	0.54583	-5.84270	5.1361E-09	1.3008E-06
K13714	588.82764	-3.11345	0.56478	-5.51266	3.5345E-08	6.5416E-06
K08659	2752.36827	-2.88077	0.41992	-6.86027	6.8728E-12	1.2879E-08
K07006	664.92748	-2.69128	0.49921	-5.39106	7.0044E-08	1.1092E-05
K02077	750.88996	-2.68393	0.47674	-5.62971	1.8051E-08	3.9483E-06
K08728	721.78538	-2.62286	0.48887	-5.36516	8.0878E-08	1.1479E-05
K02074	721.82127	-2.62286	0.48892	-5.36465	8.1105E-08	1.1479E-05
K02075	721.82127	-2.62286	0.48892	-5.36465	8.1105E-08	1.1479E-05
K05910	690.17820	-2.57935	0.50334	-5.12446	2.9840E-07	2.8915E-05
K13531	704.63115	-2.52598	0.46221	-5.46497	4.6300E-08	8.2516E-06
K02248	710.67802	-2.52048	0.46267	-5.44775	5.1012E-08	8.7668E-06
K02246	706.93440	-2.50984	0.46367	-5.41301	6.1974E-08	1.0283E-05
K06994	702.15337	-2.47131	0.50317	-4.91147	9.0396E-07	6.7582E-05
K02859	748.62318	-2.44052	0.47578	-5.12946	2.9057E-07	2.8915E-05
K03697	1517.34752	-2.41625	0.39634	-6.09647	1.0844E-09	3.4929E-07
K13678	750.49619	-2.39721	0.46342	-5.17289	2.3050E-07	2.4113E-05
K19005	1530.30557	-2.38802	0.41242	-5.79024	7.0286E-09	1.6911E-06
K18104	713.58755	-2.36576	0.45853	-5.15943	2.4770E-07	2.5361E-05
K09384	801.66672	-2.35537	0.44259	-5.32183	1.0273E-07	1.3732E-05
K18892	779.37913	-2.35215	0.44796	-5.25082	1.5142E-07	1.9175E-05
K00869	781.68238	-2.33895	0.44909	-5.20818	1.9070E-07	2.1341E-05
K00938	781.68238	-2.33895	0.44909	-5.20818	1.9070E-07	2.1341E-05
K01597	781.68238	-2.33895	0.44909	-5.20818	1.9070E-07	2.1341E-05
K01641	781.68238	-2.33895	0.44909	-5.20818	1.9070E-07	2.1341E-05
K06198	781.68238	-2.33895	0.44909	-5.20818	1.9070E-07	2.1341E-05
K10041	805.98171	-2.20974	0.43683	-5.05853	4.2250E-07	3.8360E-05
K03739	796.36422	-2.20925	0.45065	-4.90235	9.4697E-07	6.7582E-05
K14188	796.26932	-2.20925	0.45080	-4.90069	9.5503E-07	6.7582E-05
K10040	1615.96032	-2.20639	0.39643	-5.56570	2.6111E-08	5.4628E-06

DEGs d 28: NC + 1,500 FTU/kg vs. NC (184 DEGs)

GeneID	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj
K03367	800.56236	-2.20283	0.45047	-4.89009	1.0079E-06	7.0290E-05
K16169	808.28496	-2.19805	0.43767	-5.02219	5.1085E-07	4.1653E-05
K02244	808.52019	-2.19519	0.43738	-5.01902	5.1936E-07	4.1653E-05
K03095	808.52019	-2.19519	0.43738	-5.01902	5.1936E-07	4.1653E-05
K07177	808.52019	-2.19519	0.43738	-5.01902	5.1936E-07	4.1653E-05
K08987	808.52019	-2.19519	0.43738	-5.01902	5.1936E-07	4.1653E-05
K01071	817.52333	-2.16338	0.42947	-5.03733	4.7207E-07	4.1409E-05
K07707	1850.57844	-2.13979	0.38547	-5.55111	2.8386E-08	5.5453E-06
K02236	869.38302	-2.09217	0.41537	-5.03683	4.7330E-07	4.1409E-05
K18891	876.98607	-2.08378	0.45021	-4.62842	3.6846E-06	2.1889E-04
K03740	819.46658	-2.06804	0.45087	-4.58678	4.5014E-06	2.5187E-04
K06286	882.83166	-2.06293	0.40267	-5.12317	3.0045E-07	2.8915E-05
K01101	883.86239	-2.05212	0.40319	-5.08975	3.5854E-07	3.3829E-05
K09155	2032.23130	-1.97403	0.37989	-5.19635	2.0324E-07	2.2227E-05
K00158	903.82824	-1.97130	0.44248	-4.45510	8.3855E-06	4.1174E-04
K02242	859.21176	-1.92786	0.41912	-4.59981	4.2288E-06	2.4225E-04
K03293	2953.80941	-1.87588	0.35328	-5.30989	1.0969E-07	1.4266E-05
K12555	932.96494	-1.86054	0.44035	-4.22516	2.3877E-05	9.9047E-04
K03492	1824.66206	-1.84501	0.40807	-4.52134	6.1450E-06	3.2019E-04
K16209	959.22751	-1.80664	0.41958	-4.30585	1.6634E-05	7.2113E-04
K08161	2096.10253	-1.75971	0.40281	-4.36858	1.2506E-05	5.8997E-04
K02245	1433.76026	-1.69816	0.39900	-4.25608	2.0805E-05	8.7422E-04
K10039	1226.14476	-1.68908	0.35625	-4.74130	2.1235E-06	1.3270E-04
K05823	840.76101	-1.62579	0.49912	-3.25730	1.1248E-03	2.1736E-02
K12700	1244.07169	-1.56593	0.51125	-3.06296	2.1916E-03	3.5707E-02
K01259	2090.61459	-1.46882	0.36691	-4.00322	6.2485E-05	2.2272E-03
K01256	1352.01923	-1.44146	0.47516	-3.03361	2.4165E-03	3.7390E-02
K09963	1789.09990	-1.43725	0.29420	-4.88520	1.0333E-06	7.1029E-05
K05964	1270.18218	-1.42643	0.48410	-2.94657	3.2131E-03	4.6712E-02
K01215	1338.00614	-1.42595	0.33166	-4.29948	1.7120E-05	7.3554E-04
K05966	1270.32913	-1.42530	0.48407	-2.94443	3.2355E-03	4.6895E-02
K03975	1366.87037	-1.42291	0.46952	-3.03055	2.4411E-03	3.7650E-02
K03549	1605.19750	-1.39371	0.40325	-3.45623	5.4780E-04	1.2796E-02
K00867	1411.75271	-1.37135	0.44782	-3.06230	2.1964E-03	3.5707E-02
K03316	1418.42429	-1.36553	0.44762	-3.05068	2.2833E-03	3.6381E-02
K00364	1428.37351	-1.35097	0.43134	-3.13202	1.7361E-03	3.0268E-02
K07305	1423.32739	-1.34858	0.44682	-3.01814	2.5433E-03	3.8608E-02
K00851	1404.69798	-1.32478	0.44188	-2.99801	2.7175E-03	4.0864E-02
K13256	1434.61167	-1.31906	0.43341	-3.04348	2.3386E-03	3.6846E-02

DEGs d 28: NC + 1,500 FTU/kg vs. NC (184 DEGs)

GeneID	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj
K16012	1434.88807	-1.31783	0.43336	-3.04094	2.3584E-03	3.6846E-02
K16013	1434.88807	-1.31783	0.43336	-3.04094	2.3584E-03	3.6846E-02
K00135	1448.61210	-1.30855	0.43341	-3.01918	2.5346E-03	3.8608E-02
K02761	8953.04755	-1.28269	0.22735	-5.64183	1.6826E-08	3.8555E-06
K01823	1458.54432	-1.28187	0.43149	-2.97081	2.9702E-03	4.3575E-02
K07706	4356.41016	-1.21787	0.29618	-4.11191	3.9240E-05	1.5106E-03
K00980	1969.03288	-1.18386	0.38438	-3.07988	2.0708E-03	3.4430E-02
K00841	2648.21856	-1.16418	0.33966	-3.42753	6.0909E-04	1.3825E-02
K09773	4740.73048	-1.05420	0.31745	-3.32082	8.9753E-04	1.8549E-02
K01916	3398.87672	-1.02338	0.27976	-3.65807	2.5413E-04	7.5485E-03
K16951	1762.73673	1.03833	0.30174	3.44111	5.7933E-04	1.3338E-02
K00385	1762.60903	1.03846	0.30177	3.44122	5.7909E-04	1.3338E-02
K16950	1762.60903	1.03846	0.30177	3.44122	5.7909E-04	1.3338E-02
K19158	4062.55483	1.04923	0.34098	3.07705	2.0906E-03	3.4509E-02
K03300	1391.83559	1.05016	0.34250	3.06613	2.1685E-03	3.5492E-02
K01583	1472.20345	1.05372	0.33527	3.14286	1.6730E-03	2.9382E-02
K11261	1580.87661	1.05859	0.35268	3.00160	2.6856E-03	4.0512E-02
K16203	2886.85343	1.06479	0.30099	3.53759	4.0380E-04	1.1297E-02
K01849	1679.96485	1.06527	0.33935	3.13912	1.6945E-03	2.9651E-02
K01848	1590.36767	1.06672	0.35011	3.04681	2.3128E-03	3.6731E-02
K07075	9005.94569	1.07884	0.21779	4.95357	7.2866E-07	5.6553E-05
K17206	1395.53809	1.08301	0.34660	3.12468	1.7800E-03	3.0700E-02
K18344	2811.19962	1.09512	0.23573	4.64557	3.3914E-06	2.0399E-04
K08217	8384.56405	1.10492	0.18549	5.95667	2.5742E-09	7.7420E-07
K01640	2831.27453	1.11366	0.30992	3.59337	3.2643E-04	9.3498E-03
K15598	1168.73211	1.11959	0.35039	3.19524	1.3972E-03	2.5275E-02
K15599	1168.73211	1.11959	0.35039	3.19524	1.3972E-03	2.5275E-02
K15600	1168.73211	1.11959	0.35039	3.19524	1.3972E-03	2.5275E-02
K11618	1307.20097	1.13376	0.35571	3.18735	1.4358E-03	2.5664E-02
K02106	2833.13463	1.14547	0.31567	3.62870	2.8485E-04	8.3580E-03
K01844	1342.56754	1.14812	0.35359	3.24705	1.1661E-03	2.2267E-02
K18011	1342.56754	1.14812	0.35359	3.24705	1.1661E-03	2.2267E-02
K01615	4213.64861	1.14932	0.18042	6.37030	1.8865E-10	9.3202E-08
K18348	3169.32585	1.15226	0.28156	4.09242	4.2689E-05	1.6175E-03
K18122	1401.39691	1.15472	0.34770	3.32100	8.9697E-04	1.8549E-02
K17898	1263.36431	1.16715	0.36614	3.18768	1.4342E-03	2.5664E-02
K17899	1263.36431	1.16715	0.36614	3.18768	1.4342E-03	2.5664E-02
K18012	2561.37438	1.17120	0.32910	3.55879	3.7257E-04	1.0484E-02
K07654	584.02450	1.17147	0.33289	3.51907	4.3307E-04	1.1678E-02

DEGs d 28: NC + 1,500 FTU/kg vs. NC (184 DEGs)

GeneID	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj
K11528	583.89680	1.17192	0.33295	3.51975	4.3195E-04	1.1678E-02
K00034	2587.57622	1.17406	0.30884	3.80157	1.4378E-04	4.6126E-03
K01699	1308.68594	1.18637	0.35959	3.29924	9.6947E-04	1.9357E-02
K13919	1308.68594	1.18637	0.35959	3.29924	9.6947E-04	1.9357E-02
K13920	1308.68594	1.18637	0.35959	3.29924	9.6947E-04	1.9357E-02
K03303	3146.37936	1.18765	0.39811	2.98324	2.8521E-03	4.2229E-02
K14445	1603.10891	1.19262	0.33790	3.52947	4.1640E-04	1.1516E-02
K03404	1219.06704	1.19677	0.37300	3.20845	1.3345E-03	2.4604E-02
K03405	1219.06704	1.19677	0.37300	3.20845	1.3345E-03	2.4604E-02
K16875	1218.89697	1.19727	0.37306	3.20938	1.3302E-03	2.4604E-02
K17742	1218.89697	1.19727	0.37306	3.20938	1.3302E-03	2.4604E-02
K17818	1218.89697	1.19727	0.37306	3.20938	1.3302E-03	2.4604E-02
K18118	2474.96786	1.20397	0.33741	3.56830	3.5931E-04	1.0171E-02
K18345	2199.49678	1.20666	0.28177	4.28241	1.8488E-05	7.8730E-04
K01476	1256.39013	1.20895	0.37018	3.26582	1.0915E-03	2.1264E-02
K11615	3746.36883	1.21127	0.17961	6.74399	1.5410E-11	1.8538E-08
K18120	5713.09745	1.21153	0.28009	4.32543	1.5223E-05	6.7206E-04
K06859	1265.47682	1.24019	0.37140	3.33924	8.4007E-04	1.7887E-02
K06608	1687.81243	1.30556	0.37288	3.50126	4.6306E-04	1.2110E-02
K03337	2111.61065	1.34646	0.30433	4.42437	9.6724E-06	4.7014E-04
K06606	696.63292	1.34750	0.44982	2.99568	2.7384E-03	4.1050E-02
K11692	2882.17795	1.35452	0.20823	6.50490	7.7745E-11	4.6764E-08
K09133	1042.23676	1.36562	0.36482	3.74330	1.8162E-04	5.7122E-03
K16213	2845.95941	1.40757	0.44173	3.18652	1.4400E-03	2.5664E-02
K11691	2691.30633	1.40985	0.21272	6.62781	3.4070E-11	2.7324E-08
K11614	2769.29007	1.41079	0.20632	6.83803	8.0291E-12	1.2879E-08
K17735	1947.06526	1.42894	0.35513	4.02372	5.7286E-05	2.0572E-03
K12251	2749.77108	1.53399	0.43620	3.51668	4.3698E-04	1.1682E-02
K05306	1113.74331	1.55219	0.31999	4.85079	1.2297E-06	8.2185E-05
K09704	2723.48649	1.56532	0.45567	3.43519	5.9213E-04	1.3504E-02
K03335	243.06055	1.69198	0.51582	3.28017	1.0374E-03	2.0376E-02
K01028	762.65154	1.73612	0.42655	4.07015	4.6984E-05	1.7391E-03
K14654	806.68298	1.74171	0.41879	4.15888	3.1982E-05	1.2719E-03
K03430	817.86131	1.96044	0.39368	4.97984	6.3638E-07	5.0201E-05
K01906	10.56311	2.71411	0.78525	3.45637	5.4750E-04	1.2796E-02
K16651	10.80270	2.76493	0.78539	3.52045	4.3082E-04	1.1678E-02
K13820	11.82582	2.87633	0.73788	3.89808	9.6958E-05	3.3326E-03
K09706	10.38448	2.98562	0.84861	3.51824	4.3441E-04	1.1678E-02
K05967	17.93864	2.99776	0.73753	4.06456	4.8122E-05	1.7636E-03

DEGs d 28: NC + 1,500 FTU/kg vs. NC (184 DEGs)

GeneID	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj
K02191	19.20612	3.13419	0.69145	4.53277	5.8215E-06	3.1125E-04
K03399	19.17023	3.13420	0.69124	4.53415	5.7836E-06	3.1125E-04
K07062	21.98151	3.37422	1.02667	3.28656	1.0142E-03	2.0001E-02
K01551	171.39642	3.49829	1.03155	3.39131	6.9560E-04	1.5284E-02
K02626	7.39538	3.54939	1.06880	3.32091	8.9726E-04	1.8549E-02
K06027	7.39538	3.54939	1.06880	3.32091	8.9726E-04	1.8549E-02
K00356	7.06480	3.64312	1.06750	3.41276	6.4309E-04	1.4393E-02
K09126	7.10554	3.64414	1.03361	3.52564	4.2246E-04	1.1616E-02
K19114	8.42033	3.69405	0.99871	3.69883	2.1660E-04	6.5966E-03
K19115	8.49301	3.70343	0.96404	3.84158	1.2224E-04	4.0290E-03
K19116	8.49301	3.70343	0.96404	3.84158	1.2224E-04	4.0290E-03
K01896	7.30439	3.72185	1.07372	3.46631	5.2765E-04	1.2446E-02
K02482	7.30439	3.72185	1.07372	3.46631	5.2765E-04	1.2446E-02
K03727	7.30439	3.72185	1.07372	3.46631	5.2765E-04	1.2446E-02
K06937	7.30439	3.72185	1.07372	3.46631	5.2765E-04	1.2446E-02
K07469	7.30439	3.72185	1.07372	3.46631	5.2765E-04	1.2446E-02
K07669	7.30439	3.72185	1.07372	3.46631	5.2765E-04	1.2446E-02
K09928	7.30439	3.72185	1.07372	3.46631	5.2765E-04	1.2446E-02
K10747	7.30439	3.72185	1.07372	3.46631	5.2765E-04	1.2446E-02
K11180	7.30439	3.72185	1.07372	3.46631	5.2765E-04	1.2446E-02
K11181	7.30439	3.72185	1.07372	3.46631	5.2765E-04	1.2446E-02
K13572	7.30439	3.72185	1.07372	3.46631	5.2765E-04	1.2446E-02
K15495	7.30439	3.72185	1.07372	3.46631	5.2765E-04	1.2446E-02
K16885	7.30439	3.72185	1.07372	3.46631	5.2765E-04	1.2446E-02
K16886	7.30439	3.72185	1.07372	3.46631	5.2765E-04	1.2446E-02
K16887	7.30439	3.72185	1.07372	3.46631	5.2765E-04	1.2446E-02
K06873	7.34028	3.72218	1.06214	3.50442	4.5760E-04	1.2099E-02
K09942	7.34028	3.72218	1.06214	3.50442	4.5760E-04	1.2099E-02
K00394	7.34432	3.73456	1.07483	3.47456	5.1170E-04	1.2446E-02
K00395	7.34432	3.73456	1.07483	3.47456	5.1170E-04	1.2446E-02
K02664	8.74477	3.73498	0.95206	3.92306	8.7433E-05	3.0936E-03
K02663	8.78551	3.73531	0.91927	4.06332	4.8379E-05	1.7636E-03

Number of up- and down-regulated differentially expressed genes (DEGs) based on predicted metagenome of the cecal microbiota compared between YPM x Ross 708 male broilers provided a negative control (NC; 0 FTU/kg) diet and a NC with phytase supplementation (500 or 1,500 FTU/kg) on d 28 and 42.

DEGs d 42: NC + 500 FTU/kg vs. NC (1,340 DEGs)						
GeneID	**baseMean**	**log2FoldChange**	**lfcSE**	**stat**	**pvalue**	**padj**
K13893	406.23478	-2.66468	0.79081	-3.36956	7.5288E-04	2.9134E-03
K01894	406.19387	-2.66444	0.79088	-3.36895	7.5454E-04	2.9134E-03
K08219	406.19387	-2.66444	0.79088	-3.36895	7.5454E-04	2.9134E-03
K10679	406.19387	-2.66444	0.79088	-3.36895	7.5454E-04	2.9134E-03
K11744	406.19387	-2.66444	0.79088	-3.36895	7.5454E-04	2.9134E-03
K13894	406.19387	-2.66444	0.79088	-3.36895	7.5454E-04	2.9134E-03
K13895	406.19387	-2.66444	0.79088	-3.36895	7.5454E-04	2.9134E-03
K07026	406.17002	-2.66429	0.79099	-3.36832	7.5629E-04	2.9134E-03
K05523	406.98675	-2.66395	0.78668	-3.38633	7.0835E-04	2.9134E-03
K07784	406.98675	-2.66395	0.78668	-3.38633	7.0835E-04	2.9134E-03
K09771	406.98675	-2.66395	0.78668	-3.38633	7.0835E-04	2.9134E-03
K11921	406.98675	-2.66395	0.78668	-3.38633	7.0835E-04	2.9134E-03
K03676	406.85971	-2.66395	0.78704	-3.38476	7.1240E-04	2.9134E-03
K00276	406.23506	-2.66395	0.78903	-3.37624	7.3483E-04	2.9134E-03
K02616	406.23506	-2.66395	0.78903	-3.37624	7.3483E-04	2.9134E-03
K04063	406.23506	-2.66395	0.78903	-3.37624	7.3483E-04	2.9134E-03
K05710	406.23506	-2.66395	0.78903	-3.37624	7.3483E-04	2.9134E-03
K05834	406.23506	-2.66395	0.78903	-3.37624	7.3483E-04	2.9134E-03
K08173	406.23506	-2.66395	0.78903	-3.37624	7.3483E-04	2.9134E-03
K09472	406.23506	-2.66395	0.78903	-3.37624	7.3483E-04	2.9134E-03
K11074	406.23506	-2.66395	0.78903	-3.37624	7.3483E-04	2.9134E-03
K13896	406.23506	-2.66395	0.78903	-3.37624	7.3483E-04	2.9134E-03
K14052	406.23506	-2.66395	0.78903	-3.37624	7.3483E-04	2.9134E-03
K00094	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K00137	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K00146	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K00427	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K00673	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K00840	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K00932	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K01093	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K01169	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K01355	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K01467	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K01484	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03

DEGs d 42: NC + 500 FTU/kg vs. NC (1,340 DEGs)

GeneID	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj
K01521	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K01631	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K01671	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K01725	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K02024	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K02080	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K02100	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K02255	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K02317	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K02364	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K02436	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K02466	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K02467	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K02468	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K02533	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K02562	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K02615	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K02617	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K02753	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K02846	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K02848	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K02850	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K02855	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K02972	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K03119	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K03207	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K03208	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K03220	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K03275	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K03276	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K03278	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K03279	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K03280	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K03290	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K03329	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K03425	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K03445	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K03459	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K03468	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03

DEGs d 42: NC + 500 FTU/kg vs. NC (1,340 DEGs)						
GeneID	**baseMean**	**log2FoldChange**	**lfcSE**	**stat**	**pvalue**	**padj**
K03516	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K03532	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K03618	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K03619	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K03668	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K03669	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K03670	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K03672	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K03712	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K03747	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K03757	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K03759	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K03765	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K03805	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K03807	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K03809	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K03815	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K03818	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K03819	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K03836	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K03838	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K03972	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K04020	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K04021	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K04022	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K04025	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K04033	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K04064	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K04081	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K04333	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K04334	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K04335	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K04336	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K04337	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K04338	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K04775	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K05517	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K05526	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K05594	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03

DEGs d 42: NC + 500 FTU/kg vs. NC (1,340 DEGs)

GeneID	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj
K05708	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K05709	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K05711	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K05712	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K05713	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K05714	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K05774	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K05775	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K05777	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K05778	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K05779	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K05789	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K05817	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K05818	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K05819	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K05875	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K05876	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K05880	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K05887	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K05984	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K06132	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K06141	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K06155	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K06159	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K06197	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K06214	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K06447	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K06906	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K07039	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K07127	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K07229	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K07232	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K07279	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K07283	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K07290	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K07314	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K07319	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K07336	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K07354	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03

DEGs d 42: NC + 500 FTU/kg vs. NC (1,340 DEGs)						
GeneID	**baseMean**	**log2FoldChange**	**lfcSE**	**stat**	**pvalue**	**padj**
K07356	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K07358	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K07470	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K07494	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K07589	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K07592	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K07639	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K07641	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K07643	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K07644	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K07647	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K07661	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K07663	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K07665	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K07678	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K07679	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K07688	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K07690	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K07700	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K07701	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K07702	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K07703	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K07709	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K07740	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K07772	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K07781	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K07782	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K07786	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K07796	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K07797	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K07803	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K07810	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K07811	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K08137	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K08160	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K08172	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K08194	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K08225	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K08318	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03

DEGs d 42: NC + 500 FTU/kg vs. NC (1,340 DEGs)

GeneID	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj
K08319	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K08320	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K08324	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K08326	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K08349	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K08350	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K08351	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K08566	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K08723	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K08970	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K08984	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K08997	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K09016	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K09017	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K09018	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K09019	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K09021	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K09023	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K09024	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K09456	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K09470	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K09471	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K09473	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K09475	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K09477	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K09612	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K09712	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K09914	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K09915	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K09918	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K09920	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K09933	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K09934	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K09937	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K09953	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K09969	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K09970	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K09971	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K09972	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03

DEGs d 42: NC + 500 FTU/kg vs. NC (1,340 DEGs)						
GeneID	**baseMean**	**log2FoldChange**	**lfcSE**	**stat**	**pvalue**	**padj**
K09975	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K09980	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K09996	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K10013	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K10014	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K10015	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K10016	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K10017	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K10037	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K10038	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K10213	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K10538	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K10711	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K10831	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K10906	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K10973	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K10975	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K11073	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K11075	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K11106	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K11139	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K11177	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K11178	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K11192	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K11208	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K11264	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K11391	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K11392	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K11530	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K11531	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K11685	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K11732	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K11736	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K11739	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K11746	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K11922	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K11929	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K11930	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K11934	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03

DEGs d 42: NC + 500 FTU/kg vs. NC (1,340 DEGs)

GeneID	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj
K11935	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K11938	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K11941	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K12068	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K12112	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K12113	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K12147	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K12151	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K12262	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K12264	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K12265	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K12266	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K12290	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K12291	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K12299	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K12528	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K12529	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K12784	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K12785	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K12786	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K12787	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K12788	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K12789	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K12790	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K12810	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K12943	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K12944	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K12945	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K12957	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K13243	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K13255	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K13480	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K13483	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K13574	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K13620	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K13630	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K13631	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K13632	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K13635	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03

DEGs d 42: NC + 500 FTU/kg vs. NC (1,340 DEGs)						
GeneID	**baseMean**	**log2FoldChange**	**lfcSE**	**stat**	**pvalue**	**padj**
K13637	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K13650	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K13654	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K13683	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K13684	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K13890	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K13891	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K13892	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K13938	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K14051	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K14053	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K14056	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K14057	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K14060	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K14062	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K14063	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K14064	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K14287	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K14348	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K14414	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K14591	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K14762	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K15268	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K15368	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K15540	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K15548	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K15550	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K15551	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K15722	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K15735	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K15737	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K15827	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K15828	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K15829	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K15830	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K15831	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K15832	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K15833	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K15834	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03

DEGs d 42: NC + 500 FTU/kg vs. NC (1,340 DEGs)						
GeneID	**baseMean**	**log2FoldChange**	**lfcSE**	**stat**	**pvalue**	**padj**
K15836	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K16041	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K16042	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K16088	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K16090	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K16135	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K16136	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K16140	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K16291	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K16321	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K16324	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K16325	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K16326	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K16346	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K16347	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K16348	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K16695	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K16703	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K16710	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K16711	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K17473	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K17938	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K17948	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K18140	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K18141	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K18142	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K18326	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K18478	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K18479	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K18530	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K18531	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K18697	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K18698	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K18765	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K18841	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K18842	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K18898	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K18899	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K18921	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03

DEGs d 42: NC + 500 FTU/kg vs. NC (1,340 DEGs)

GeneID	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj
K19046	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K19125	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K19148	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K19155	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K19234	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K19235	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K19238	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K19303	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K19334	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K19335	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K19336	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K19338	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K19353	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K19354	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K19611	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K19688	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K19709	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K19775	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K19776	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K19777	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K19778	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K19779	406.11390	-2.66395	0.79096	-3.36798	7.5720E-04	2.9134E-03
K01061	812.22779	-2.66378	0.76658	-3.47486	5.1112E-04	2.9134E-03
K03482	812.22779	-2.66378	0.76658	-3.47486	5.1112E-04	2.9134E-03
K06884	812.22779	-2.66378	0.76658	-3.47486	5.1112E-04	2.9134E-03
K06946	812.22779	-2.66378	0.76658	-3.47486	5.1112E-04	2.9134E-03
K08348	812.22779	-2.66378	0.76658	-3.47486	5.1112E-04	2.9134E-03
K09824	812.22779	-2.66378	0.76658	-3.47486	5.1112E-04	2.9134E-03
K10124	812.22779	-2.66378	0.76658	-3.47486	5.1112E-04	2.9134E-03
K11200	812.22779	-2.66378	0.76658	-3.47486	5.1112E-04	2.9134E-03
K13743	812.22779	-2.66378	0.76658	-3.47486	5.1112E-04	2.9134E-03
K16076	812.22779	-2.66378	0.76658	-3.47486	5.1112E-04	2.9134E-03
K16692	812.22779	-2.66378	0.76658	-3.47486	5.1112E-04	2.9134E-03
K18324	812.22779	-2.66378	0.76658	-3.47486	5.1112E-04	2.9134E-03
K18920	812.22779	-2.66378	0.76658	-3.47486	5.1112E-04	2.9134E-03
K19126	812.22779	-2.66378	0.76658	-3.47486	5.1112E-04	2.9134E-03
K03291	1218.46285	-2.66372	0.75311	-3.53697	4.0474E-04	2.9134E-03
K03755	1218.34169	-2.66372	0.75376	-3.53390	4.0947E-04	2.9134E-03
K07493	1218.34169	-2.66372	0.75376	-3.53390	4.0947E-04	2.9134E-03

DEGs d 42: NC + 500 FTU/kg vs. NC (1,340 DEGs)

GeneID	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj
K11201	1218.34169	-2.66372	0.75376	-3.53390	4.0947E-04	2.9134E-03
K18919	1218.34169	-2.66372	0.75376	-3.53390	4.0947E-04	2.9134E-03
K18922	1218.34169	-2.66372	0.75376	-3.53390	4.0947E-04	2.9134E-03
K12678	1624.45558	-2.66367	0.81122	-3.28352	1.0252E-03	3.7706E-03
K12687	1624.45558	-2.66367	0.81122	-3.28352	1.0252E-03	3.7706E-03
K18838	1624.45558	-2.66367	0.81122	-3.28352	1.0252E-03	3.7706E-03
K01160	2436.68338	-2.66364	0.81114	-3.28382	1.0241E-03	3.7706E-03
K07480	2436.68338	-2.66364	0.81114	-3.28382	1.0241E-03	3.7706E-03
K18862	2842.79727	-2.66363	0.81112	-3.28391	1.0238E-03	3.7706E-03
K15536	1218.51855	-2.66329	0.75381	-3.53312	4.1069E-04	2.9134E-03
K04018	812.40466	-2.66314	0.76665	-3.47371	5.1331E-04	2.9134E-03
K14261	406.34689	-2.66301	0.79113	-3.36611	7.6237E-04	2.9134E-03
K07246	406.39037	-2.66300	0.79074	-3.36773	7.5789E-04	2.9134E-03
K01692	812.59149	-2.66283	0.76468	-3.48227	4.9718E-04	2.9134E-03
K10108	407.16361	-2.66268	0.78682	-3.38410	7.1412E-04	2.9134E-03
K00101	406.41193	-2.66267	0.78917	-3.37403	7.4077E-04	2.9134E-03
K13979	406.41193	-2.66267	0.78917	-3.37403	7.4077E-04	2.9134E-03
K15034	406.41193	-2.66267	0.78917	-3.37403	7.4077E-04	2.9134E-03
K01908	406.29076	-2.66267	0.79110	-3.36577	7.6329E-04	2.9134E-03
K02196	406.29076	-2.66267	0.79110	-3.36577	7.6329E-04	2.9134E-03
K02199	406.29076	-2.66267	0.79110	-3.36577	7.6329E-04	2.9134E-03
K02200	406.29076	-2.66267	0.79110	-3.36577	7.6329E-04	2.9134E-03
K02430	406.29076	-2.66267	0.79110	-3.36577	7.6329E-04	2.9134E-03
K02506	406.29076	-2.66267	0.79110	-3.36577	7.6329E-04	2.9134E-03
K02568	406.29076	-2.66267	0.79110	-3.36577	7.6329E-04	2.9134E-03
K02569	406.29076	-2.66267	0.79110	-3.36577	7.6329E-04	2.9134E-03
K02572	406.29076	-2.66267	0.79110	-3.36577	7.6329E-04	2.9134E-03
K02681	406.29076	-2.66267	0.79110	-3.36577	7.6329E-04	2.9134E-03
K03078	406.29076	-2.66267	0.79110	-3.36577	7.6329E-04	2.9134E-03
K03304	406.29076	-2.66267	0.79110	-3.36577	7.6329E-04	2.9134E-03
K03477	406.29076	-2.66267	0.79110	-3.36577	7.6329E-04	2.9134E-03
K03675	406.29076	-2.66267	0.79110	-3.36577	7.6329E-04	2.9134E-03
K04013	406.29076	-2.66267	0.79110	-3.36577	7.6329E-04	2.9134E-03
K04014	406.29076	-2.66267	0.79110	-3.36577	7.6329E-04	2.9134E-03
K04015	406.29076	-2.66267	0.79110	-3.36577	7.6329E-04	2.9134E-03
K04016	406.29076	-2.66267	0.79110	-3.36577	7.6329E-04	2.9134E-03
K04017	406.29076	-2.66267	0.79110	-3.36577	7.6329E-04	2.9134E-03
K07147	406.29076	-2.66267	0.79110	-3.36577	7.6329E-04	2.9134E-03
K07261	406.29076	-2.66267	0.79110	-3.36577	7.6329E-04	2.9134E-03

DEGs d 42: NC + 500 FTU/kg vs. NC (1,340 DEGs)

GeneID	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj
K07262	406.29076	-2.66267	0.79110	-3.36577	7.6329E-04	2.9134E-03
K07666	406.29076	-2.66267	0.79110	-3.36577	7.6329E-04	2.9134E-03
K07674	406.29076	-2.66267	0.79110	-3.36577	7.6329E-04	2.9134E-03
K07812	406.29076	-2.66267	0.79110	-3.36577	7.6329E-04	2.9134E-03
K07821	406.29076	-2.66267	0.79110	-3.36577	7.6329E-04	2.9134E-03
K09909	406.29076	-2.66267	0.79110	-3.36577	7.6329E-04	2.9134E-03
K10109	406.29076	-2.66267	0.79110	-3.36577	7.6329E-04	2.9134E-03
K10110	406.29076	-2.66267	0.79110	-3.36577	7.6329E-04	2.9134E-03
K10111	406.29076	-2.66267	0.79110	-3.36577	7.6329E-04	2.9134E-03
K11745	406.29076	-2.66267	0.79110	-3.36577	7.6329E-04	2.9134E-03
K13283	406.29076	-2.66267	0.79110	-3.36577	7.6329E-04	2.9134E-03
K17247	406.29076	-2.66267	0.79110	-3.36577	7.6329E-04	2.9134E-03
K01087	406.41255	-2.66240	0.78908	-3.37408	7.4063E-04	2.9134E-03
K07119	407.10812	-2.66206	0.78670	-3.38382	7.1485E-04	2.9134E-03
K05522	406.47759	-2.66206	0.78729	-3.38129	7.2145E-04	2.9134E-03
K06048	406.47759	-2.66206	0.78729	-3.38129	7.2145E-04	2.9134E-03
K00138	406.35643	-2.66206	0.78905	-3.37374	7.4153E-04	2.9134E-03
K00697	406.35643	-2.66206	0.78905	-3.37374	7.4153E-04	2.9134E-03
K02609	406.35643	-2.66206	0.78905	-3.37374	7.4153E-04	2.9134E-03
K02610	406.35643	-2.66206	0.78905	-3.37374	7.4153E-04	2.9134E-03
K02611	406.35643	-2.66206	0.78905	-3.37374	7.4153E-04	2.9134E-03
K02612	406.35643	-2.66206	0.78905	-3.37374	7.4153E-04	2.9134E-03
K02613	406.35643	-2.66206	0.78905	-3.37374	7.4153E-04	2.9134E-03
K02618	406.35643	-2.66206	0.78905	-3.37374	7.4153E-04	2.9134E-03
K09781	406.35643	-2.66206	0.78905	-3.37374	7.4153E-04	2.9134E-03
K11738	406.35643	-2.66206	0.78905	-3.37374	7.4153E-04	2.9134E-03
K01659	406.23526	-2.66206	0.79099	-3.36549	7.6408E-04	2.9134E-03
K03919	406.23526	-2.66206	0.79099	-3.36549	7.6408E-04	2.9134E-03
K05835	406.23526	-2.66206	0.79099	-3.36549	7.6408E-04	2.9134E-03
K07393	406.23526	-2.66206	0.79099	-3.36549	7.6408E-04	2.9134E-03
K11900	1219.98503	-2.66197	0.75210	-3.53937	4.0109E-04	2.9134E-03
K18837	1219.98503	-2.66197	0.75210	-3.53937	4.0109E-04	2.9134E-03
K13069	812.60428	-2.66121	0.76481	-3.47958	5.0220E-04	2.9134E-03
K16301	814.86515	-2.66116	0.76089	-3.49742	4.6978E-04	2.9134E-03
K03221	813.87113	-2.66116	0.76415	-3.48250	4.9676E-04	2.9134E-03
K03229	813.87113	-2.66116	0.76415	-3.48250	4.9676E-04	2.9134E-03
K06078	813.87113	-2.66116	0.76415	-3.48250	4.9676E-04	2.9134E-03
K07274	813.87113	-2.66116	0.76415	-3.48250	4.9676E-04	2.9134E-03
K07285	813.87113	-2.66116	0.76415	-3.48250	4.9676E-04	2.9134E-03

DEGs d 42: NC + 500 FTU/kg vs. NC (1,340 DEGs)

GeneID	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj
K07348	813.87113	-2.66116	0.76415	-3.48250	4.9676E-04	2.9134E-03
K09476	813.87113	-2.66116	0.76415	-3.48250	4.9676E-04	2.9134E-03
K11209	813.87113	-2.66116	0.76415	-3.48250	4.9676E-04	2.9134E-03
K11891	813.87113	-2.66116	0.76415	-3.48250	4.9676E-04	2.9134E-03
K11892	813.87113	-2.66116	0.76415	-3.48250	4.9676E-04	2.9134E-03
K11893	813.87113	-2.66116	0.76415	-3.48250	4.9676E-04	2.9134E-03
K11895	813.87113	-2.66116	0.76415	-3.48250	4.9676E-04	2.9134E-03
K11901	813.87113	-2.66116	0.76415	-3.48250	4.9676E-04	2.9134E-03
K11905	813.87113	-2.66116	0.76415	-3.48250	4.9676E-04	2.9134E-03
K11906	813.87113	-2.66116	0.76415	-3.48250	4.9676E-04	2.9134E-03
K11907	813.87113	-2.66116	0.76415	-3.48250	4.9676E-04	2.9134E-03
K11910	813.87113	-2.66116	0.76415	-3.48250	4.9676E-04	2.9134E-03
K11911	813.87113	-2.66116	0.76415	-3.48250	4.9676E-04	2.9134E-03
K12056	813.87113	-2.66116	0.76415	-3.48250	4.9676E-04	2.9134E-03
K12063	813.87113	-2.66116	0.76415	-3.48250	4.9676E-04	2.9134E-03
K12139	813.87113	-2.66116	0.76415	-3.48250	4.9676E-04	2.9134E-03
K13735	813.87113	-2.66116	0.76415	-3.48250	4.9676E-04	2.9134E-03
K16322	813.87113	-2.66116	0.76415	-3.48250	4.9676E-04	2.9134E-03
K18828	813.87113	-2.66116	0.76415	-3.48250	4.9676E-04	2.9134E-03
K00389	406.62244	-2.66079	0.78740	-3.37919	7.2700E-04	2.9134E-03
K09780	406.38010	-2.66078	0.79110	-3.36340	7.6989E-04	2.9228E-03
K14744	2849.37062	-2.66064	0.80807	-3.29259	9.9268E-04	3.6727E-03
K03503	406.21462	-2.66055	0.79109	-3.36315	7.7057E-04	2.9228E-03
K06143	406.21462	-2.66055	0.79109	-3.36315	7.7057E-04	2.9228E-03
K06149	814.04799	-2.66052	0.76422	-3.48135	4.9890E-04	2.9134E-03
K07347	3257.12785	-2.66036	0.80780	-3.29336	9.8999E-04	3.6658E-03
K05782	406.47384	-2.66035	0.78906	-3.37152	7.4754E-04	2.9134E-03
K07248	406.48541	-2.66031	0.79070	-3.36449	7.6685E-04	2.9214E-03
K00370	814.86535	-2.66022	0.76190	-3.49158	4.8018E-04	2.9134E-03
K00371	814.86535	-2.66022	0.76190	-3.49158	4.8018E-04	2.9134E-03
K00374	814.86535	-2.66022	0.76190	-3.49158	4.8018E-04	2.9134E-03
K00373	813.99249	-2.66022	0.76417	-3.48121	4.9916E-04	2.9134E-03
K03746	1221.80523	-2.65981	0.75069	-3.54317	3.9535E-04	2.9134E-03
K06211	408.39211	-2.65965	0.78554	-3.38575	7.0985E-04	2.9134E-03
K15461	408.39211	-2.65965	0.78554	-3.38575	7.0985E-04	2.9134E-03
K00380	408.71006	-2.65922	0.78203	-3.40039	6.7289E-04	2.9134E-03
K00381	407.83721	-2.65921	0.78636	-3.38169	7.2041E-04	2.9134E-03
K00835	407.83721	-2.65921	0.78636	-3.38169	7.2041E-04	2.9134E-03
K05939	407.83721	-2.65921	0.78636	-3.38169	7.2041E-04	2.9134E-03

DEGs d 42: NC + 500 FTU/kg vs. NC (1,340 DEGs)

GeneID	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj
K12339	407.81336	-2.65907	0.78646	-3.38105	7.2210E-04	2.9134E-03
K02192	407.79814	-2.65897	0.78637	-3.38133	7.2135E-04	2.9134E-03
K09946	406.56255	-2.65888	0.78883	-3.37066	7.4987E-04	2.9134E-03
K10985	406.49038	-2.65882	0.78756	-3.37600	7.3547E-04	2.9134E-03
K10986	406.49038	-2.65882	0.78756	-3.37600	7.3547E-04	2.9134E-03
K19123	406.49038	-2.65882	0.78756	-3.37600	7.3547E-04	2.9134E-03
K07224	408.75125	-2.65873	0.78015	-3.40798	6.5446E-04	2.9134E-03
K07243	408.75125	-2.65873	0.78015	-3.40798	6.5446E-04	2.9134E-03
K00363	408.63009	-2.65873	0.78212	-3.39941	6.7532E-04	2.9134E-03
K02298	408.63009	-2.65873	0.78212	-3.39941	6.7532E-04	2.9134E-03
K02299	408.63009	-2.65873	0.78212	-3.39941	6.7532E-04	2.9134E-03
K03535	408.63009	-2.65873	0.78212	-3.39941	6.7532E-04	2.9134E-03
K13639	407.99956	-2.65873	0.78271	-3.39682	6.8175E-04	2.9134E-03
K01497	407.87840	-2.65873	0.78449	-3.38912	7.0117E-04	2.9134E-03
K06916	407.87840	-2.65873	0.78449	-3.38912	7.0117E-04	2.9134E-03
K19304	407.87840	-2.65873	0.78449	-3.38912	7.0117E-04	2.9134E-03
K00117	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K00322	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K00855	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K00892	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K00906	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K01146	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K01150	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K01460	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K01584	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K01690	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K01782	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K01825	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K02079	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K02167	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K02182	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K02297	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K02336	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K02381	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K02386	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K02391	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K02399	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K02402	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K02403	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03

DEGs d 42: NC + 500 FTU/kg vs. NC (1,340 DEGs)

GeneID	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj
K02423	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K02425	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K02471	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K02485	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K02565	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K02844	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K03112	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K03219	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K03222	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K03225	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K03226	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K03227	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K03228	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K03230	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K03414	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K03472	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K03485	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K03567	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K03577	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K03623	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K03658	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K03717	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K03749	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K03774	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K03776	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K03777	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K03796	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K03812	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K03840	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K03970	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K03971	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K03974	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K04046	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K04062	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K04752	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K05245	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K05368	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K05591	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K05785	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03

DEGs d 42: NC + 500 FTU/kg vs. NC (1,340 DEGs)						
GeneID	**baseMean**	**log2FoldChange**	**lfcSE**	**stat**	**pvalue**	**padj**
K05790	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K05798	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K05800	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K05805	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K05811	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K05812	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K05839	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K05874	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K05877	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K05881	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K05997	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K06006	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K06073	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K06074	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K06079	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K06080	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K06144	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K06192	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K06219	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K06222	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K06445	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K06858	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K06938	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K07019	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K07070	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K07136	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K07165	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K07251	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K07269	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K07287	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K07289	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K07337	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K07350	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K07357	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K07454	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K07459	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K07490	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K07637	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K07638	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03

DEGs d 42: NC + 500 FTU/kg vs. NC (1,340 DEGs)

GeneID	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj
K07659	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K07660	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K07664	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K07673	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K07675	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K07676	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K07677	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K07684	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K07686	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K07687	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K07689	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K07708	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K07711	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K07712	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K07715	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K07771	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K07788	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K07789	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K07799	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K07804	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K07806	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K08154	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K08162	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K08163	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K08227	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K08276	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K08277	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K08279	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K08297	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K08299	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K08308	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K08313	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K08315	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K08353	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K08484	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K08485	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K08682	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K08993	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K08994	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03

DEGs d 42: NC + 500 FTU/kg vs. NC (1,340 DEGs)						
GeneID	**baseMean**	**log2FoldChange**	**lfcSE**	**stat**	**pvalue**	**padj**
K09161	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K09823	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K09857	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K09912	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K09916	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K09921	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K10001	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K10002	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K10003	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K10004	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K10673	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K10857	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K10972	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K11066	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K11734	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K11742	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K11743	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K11750	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K11894	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K11909	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K11923	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K11925	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K11926	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K11933	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K11940	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K12060	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K12061	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K12062	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K12064	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K12066	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K12067	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K12072	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K12138	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K12142	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K12145	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K12146	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K12148	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K12288	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K12289	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03

DEGs d 42: NC + 500 FTU/kg vs. NC (1,340 DEGs)						
GeneID	**baseMean**	**log2FoldChange**	**lfcSE**	**stat**	**pvalue**	**padj**
K12500	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K12525	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K12961	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K12962	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K12963	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K12972	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K12973	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K12974	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K12975	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K13014	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K13051	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K13053	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K13301	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K13629	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K13638	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K13926	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K14055	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K14588	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K15547	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K15549	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K15723	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K15736	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K15773	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K15835	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K15974	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K16074	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K16078	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K16693	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K17713	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K18015	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K18446	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K18657	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K18778	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K18824	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K18843	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K18889	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K18890	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K18923	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K18988	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03

DEGs d 42: NC + 500 FTU/kg vs. NC (1,340 DEGs)

GeneID	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj
K18997	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K19000	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K19162	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K19168	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K19236	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K19337	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K19745	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K19789	407.75723	-2.65873	0.78644	-3.38071	7.2298E-04	2.9134E-03
K12140	408.87904	-2.65861	0.78488	-3.38728	7.0591E-04	2.9134E-03
K12141	408.87904	-2.65861	0.78488	-3.38728	7.0591E-04	2.9134E-03
K19270	815.75680	-2.65856	0.76019	-3.49725	4.7009E-04	2.9134E-03
K07000	815.51447	-2.65856	0.76212	-3.48838	4.8596E-04	2.9134E-03
K07349	815.51447	-2.65856	0.76212	-3.48838	4.8596E-04	2.9134E-03
K11896	815.51447	-2.65856	0.76212	-3.48838	4.8596E-04	2.9134E-03
K12149	815.51447	-2.65856	0.76212	-3.48838	4.8596E-04	2.9134E-03
K18829	815.51447	-2.65856	0.76212	-3.48838	4.8596E-04	2.9134E-03
K00799	1223.27170	-2.65850	0.74933	-3.54781	3.8845E-04	2.9134E-03
K07346	1631.02893	-2.65845	0.80628	-3.29719	9.7658E-04	3.6192E-03
K04765	408.07020	-2.65828	0.78652	-3.37981	7.2535E-04	2.9134E-03
K01414	408.05498	-2.65819	0.78642	-3.38010	7.2461E-04	2.9134E-03
K03562	408.04635	-2.65814	0.78662	-3.37917	7.2705E-04	2.9134E-03
K01792	408.01407	-2.65794	0.78649	-3.37948	7.2623E-04	2.9134E-03
K05541	408.01407	-2.65794	0.78649	-3.37948	7.2623E-04	2.9134E-03
K06077	408.01407	-2.65794	0.78649	-3.37948	7.2623E-04	2.9134E-03
K06181	408.01407	-2.65794	0.78649	-3.37948	7.2623E-04	2.9134E-03
K06195	408.01407	-2.65794	0.78649	-3.37948	7.2623E-04	2.9134E-03
K08311	408.01407	-2.65794	0.78649	-3.37948	7.2623E-04	2.9134E-03
K09954	408.01407	-2.65794	0.78649	-3.37948	7.2623E-04	2.9134E-03
K10806	408.01407	-2.65794	0.78649	-3.37948	7.2623E-04	2.9134E-03
K14682	408.01407	-2.65794	0.78649	-3.37948	7.2623E-04	2.9134E-03
K03808	815.69133	-2.65792	0.76219	-3.48723	4.8806E-04	2.9134E-03
K05802	815.69133	-2.65792	0.76219	-3.48723	4.8806E-04	2.9134E-03
K03634	407.99022	-2.65780	0.78660	-3.37884	7.2793E-04	2.9134E-03
K00242	408.05526	-2.65746	0.78463	-3.38690	7.0686E-04	2.9134E-03
K03286	408.05526	-2.65746	0.78463	-3.38690	7.0686E-04	2.9134E-03
K07140	408.05526	-2.65746	0.78463	-3.38690	7.0686E-04	2.9134E-03
K00228	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K00245	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K00246	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03

DEGs d 42: NC + 500 FTU/kg vs. NC (1,340 DEGs)						
GeneID	**baseMean**	**log2FoldChange**	**lfcSE**	**stat**	**pvalue**	**padj**
K00247	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K00631	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K00647	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K00769	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K00884	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K00885	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K00998	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K01139	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K01141	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K01147	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K01175	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K01407	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K01682	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K01716	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K01766	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K02062	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K02063	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K02064	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K02339	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K02344	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K02439	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K02494	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K02498	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K02504	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K02505	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K02507	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K02521	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K02560	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K02679	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K02682	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K02686	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K02742	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K02847	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K02849	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K02853	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K03071	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K03089	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K03181	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K03184	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03

DEGs d 42: NC + 500 FTU/kg vs. NC (1,340 DEGs)						
GeneID	**baseMean**	**log2FoldChange**	**lfcSE**	**stat**	**pvalue**	**padj**
K03185	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K03212	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K03214	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K03314	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K03528	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K03548	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K03557	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K03566	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K03573	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K03576	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K03580	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K03582	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K03583	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K03586	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K03591	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K03597	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K03598	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K03599	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K03600	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K03603	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K03607	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K03632	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K03633	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K03643	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K03645	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K03651	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K03656	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K03673	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K03674	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K03683	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K03690	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K03720	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K03732	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K03745	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K03764	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K03788	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K03804	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K03806	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K03831	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03

DEGs d 42: NC + 500 FTU/kg vs. NC (1,340 DEGs)

GeneID	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj
K03834	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K03835	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K04044	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K04067	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K04082	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K04568	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K04691	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K04753	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K04755	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K04760	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K04770	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K04774	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K05365	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K05501	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K05527	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K05539	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K05590	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K05803	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K05809	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K05851	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K06039	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K06175	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K06186	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K06189	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K06190	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K06202	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K06203	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K06205	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K06212	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K06866	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K06879	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K06899	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K06918	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K06957	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K06968	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K07014	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K07121	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K07122	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K07153	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03

DEGs d 42: NC + 500 FTU/kg vs. NC (1,340 DEGs)						
GeneID	**baseMean**	**log2FoldChange**	**lfcSE**	**stat**	**pvalue**	**padj**
K07184	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K07186	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K07235	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K07236	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K07237	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K07275	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K07278	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K07286	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K07320	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K07340	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K07390	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K07479	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K07640	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K07645	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K07648	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K07662	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K07685	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K07724	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K07733	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K07751	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K07773	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K08159	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K08304	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K08305	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K08306	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K08312	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K08990	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K09158	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K09159	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K09160	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K09800	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K09801	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K09802	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K09862	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K09889	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K09890	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K09891	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K09893	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K09894	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03

DEGs d 42: NC + 500 FTU/kg vs. NC (1,340 DEGs)

GeneID	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj
K09895	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K09896	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K09897	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K09898	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K09899	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K09900	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K09901	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K09902	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K09904	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K09906	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K09907	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K09908	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K09910	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K09917	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K09923	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K09978	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K09997	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K09998	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K09999	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K10000	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K10763	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K11258	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K11719	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K11747	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K11751	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K12152	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K12368	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K12369	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K12370	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K12371	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K12372	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K12582	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K13643	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K13695	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K14058	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K15257	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K15396	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K15539	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K15545	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03

DEGs d 42: NC + 500 FTU/kg vs. NC (1,340 DEGs)						
GeneID	**baseMean**	**log2FoldChange**	**lfcSE**	**stat**	**pvalue**	**padj**
K15724	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K15984	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K16066	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K16092	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K16137	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K16704	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K18800	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K18850	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K19226	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K19227	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K19228	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K19229	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K19230	407.93410	-2.65745	0.78658	-3.37850	7.2882E-04	2.9134E-03
K03579	408.07974	-2.65733	0.78443	-3.38761	7.0504E-04	2.9134E-03
K14347	407.95858	-2.65733	0.78638	-3.37920	7.2696E-04	2.9134E-03
K01058	408.00738	-2.65699	0.78615	-3.37975	7.2552E-04	2.9134E-03
K11737	409.74547	-2.65686	0.77681	-3.42022	6.2571E-04	2.9134E-03
K00130	408.99379	-2.65686	0.77838	-3.41331	6.4179E-04	2.9134E-03
K00108	408.75145	-2.65685	0.78214	-3.39690	6.8153E-04	2.9134E-03
K07245	408.12093	-2.65685	0.78274	-3.39432	6.8800E-04	2.9134E-03
K19577	408.12093	-2.65685	0.78274	-3.39432	6.8800E-04	2.9134E-03
K05596	407.99976	-2.65685	0.78451	-3.38663	7.0757E-04	2.9134E-03
K07054	407.99976	-2.65685	0.78451	-3.38663	7.0757E-04	2.9134E-03
K08281	407.99976	-2.65685	0.78451	-3.38663	7.0757E-04	2.9134E-03
K10805	407.99976	-2.65685	0.78451	-3.38663	7.0757E-04	2.9134E-03
K03087	407.87860	-2.65685	0.78646	-3.37823	7.2955E-04	2.9134E-03
K08224	407.87860	-2.65685	0.78646	-3.37823	7.2955E-04	2.9134E-03
K07345	2449.83007	-2.65669	0.80488	-3.30071	9.6439E-04	3.5802E-03
K02558	408.26422	-2.65620	0.78620	-3.37852	7.2878E-04	2.9134E-03
K13821	408.34575	-2.65607	0.78276	-3.39319	6.9083E-04	2.9134E-03
K03578	408.22458	-2.65607	0.78454	-3.38551	7.1046E-04	2.9134E-03
K06887	817.15780	-2.65596	0.76039	-3.49288	4.7784E-04	2.9134E-03
K11903	817.15780	-2.65596	0.76039	-3.49288	4.7784E-04	2.9134E-03
K00982	408.20073	-2.65592	0.78465	-3.38486	7.1214E-04	2.9134E-03
K00990	408.07957	-2.65592	0.78660	-3.37647	7.3423E-04	2.9134E-03
K09791	408.07957	-2.65592	0.78660	-3.37647	7.3423E-04	2.9134E-03
K02852	408.18424	-2.65572	0.78629	-3.37754	7.3137E-04	2.9134E-03
K05787	408.18424	-2.65572	0.78629	-3.37754	7.3137E-04	2.9134E-03
K00163	408.14461	-2.65558	0.78462	-3.38453	7.1301E-04	2.9134E-03

DEGs d 42: NC + 500 FTU/kg vs. NC (1,340 DEGs)

GeneID	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj
K01354	408.14461	-2.65558	0.78462	-3.38453	7.1301E-04	2.9134E-03
K05838	408.14461	-2.65558	0.78462	-3.38453	7.1301E-04	2.9134E-03
K06980	408.14461	-2.65558	0.78462	-3.38453	7.1301E-04	2.9134E-03
K09858	408.14461	-2.65558	0.78462	-3.38453	7.1301E-04	2.9134E-03
K13288	408.14461	-2.65558	0.78462	-3.38453	7.1301E-04	2.9134E-03
K13788	408.14461	-2.65558	0.78462	-3.38453	7.1301E-04	2.9134E-03
K13498	408.02344	-2.65558	0.78657	-3.37613	7.3512E-04	2.9134E-03
K09806	408.00124	-2.65520	0.78666	-3.37529	7.3739E-04	2.9134E-03
K11939	408.11717	-2.65514	0.78452	-3.38441	7.1331E-04	2.9134E-03
K07115	408.03482	-2.65408	0.78670	-3.37368	7.4171E-04	2.9134E-03
K03756	408.13372	-2.65362	0.78394	-3.38497	7.1186E-04	2.9134E-03
K03824	408.98096	-2.65361	0.78195	-3.39360	6.8980E-04	2.9134E-03
K02345	409.40057	-2.65354	0.78319	-3.38813	7.0370E-04	2.9134E-03
K03435	409.40057	-2.65354	0.78319	-3.38813	7.0370E-04	2.9134E-03
K04080	409.40057	-2.65354	0.78319	-3.38813	7.0370E-04	2.9134E-03
K07264	409.40057	-2.65354	0.78319	-3.38813	7.0370E-04	2.9134E-03
K07351	409.40057	-2.65354	0.78319	-3.38813	7.0370E-04	2.9134E-03
K08354	409.40057	-2.65354	0.78319	-3.38813	7.0370E-04	2.9134E-03
K10748	409.40057	-2.65354	0.78319	-3.38813	7.0370E-04	2.9134E-03
K11107	409.40057	-2.65354	0.78319	-3.38813	7.0370E-04	2.9134E-03
K11250	409.40057	-2.65354	0.78319	-3.38813	7.0370E-04	2.9134E-03
K11932	409.40057	-2.65354	0.78319	-3.38813	7.0370E-04	2.9134E-03
K18476	409.40057	-2.65354	0.78319	-3.38813	7.0370E-04	2.9134E-03
K19160	409.40057	-2.65354	0.78319	-3.38813	7.0370E-04	2.9134E-03
K07642	409.42726	-2.65309	0.78797	-3.36701	7.5987E-04	2.9134E-03
K03289	406.71933	-2.65273	0.78874	-3.36326	7.7028E-04	2.9228E-03
K09794	409.63356	-2.65261	0.78335	-3.38626	7.0852E-04	2.9134E-03
K08992	408.31058	-2.65235	0.78408	-3.38275	7.1763E-04	2.9134E-03
K03554	409.57743	-2.65227	0.78332	-3.38593	7.0938E-04	2.9134E-03
K03981	409.57743	-2.65227	0.78332	-3.38593	7.0938E-04	2.9134E-03
K09892	409.57743	-2.65227	0.78332	-3.38593	7.0938E-04	2.9134E-03
K09911	409.57743	-2.65227	0.78332	-3.38593	7.0938E-04	2.9134E-03
K13634	409.57743	-2.65227	0.78332	-3.38593	7.0938E-04	2.9134E-03
K05589	408.19011	-2.65217	0.78683	-3.37068	7.4982E-04	2.9134E-03
K07156	408.54215	-2.65123	0.78114	-3.39406	6.8863E-04	2.9134E-03
K02841	409.08529	-2.64969	0.78176	-3.38941	7.0043E-04	2.9134E-03
K02168	409.81621	-2.64871	0.78335	-3.38125	7.2156E-04	2.9134E-03
K00362	409.39491	-2.64538	0.77962	-3.39316	6.9090E-04	2.9134E-03
K03077	406.92713	-2.64486	0.79166	-3.34091	8.3505E-04	3.1401E-03

DEGs d 42: NC + 500 FTU/kg vs. NC (1,340 DEGs)

GeneID	**baseMean**	**log2FoldChange**	**lfcSE**	**stat**	**pvalue**	**padj**
K19156	408.10852	-2.64413	0.79041	-3.34526	8.2205E-04	3.0938E-03
K05804	412.68724	-2.64327	0.77892	-3.39348	6.9010E-04	2.9134E-03
K12143	819.95570	-2.64083	0.75350	-3.50477	4.5701E-04	2.9134E-03
K02575	820.07706	-2.63990	0.75351	-3.50349	4.5921E-04	2.9134E-03
K14061	414.33058	-2.63819	0.77752	-3.39306	6.9117E-04	2.9134E-03
K00313	1230.89896	-2.63342	0.73857	-3.56557	3.6306E-04	2.9134E-03
K16091	407.23375	-2.63130	0.79012	-3.33024	8.6770E-04	3.2572E-03
K00428	407.75665	-2.62676	0.78974	-3.32612	8.8063E-04	3.3001E-03
K06140	410.62095	-2.62500	0.77120	-3.40380	6.6456E-04	2.9134E-03
K00112	410.72284	-2.62375	0.77133	-3.40158	6.6998E-04	2.9134E-03
K00113	410.72284	-2.62375	0.77133	-3.40158	6.6998E-04	2.9134E-03
K13888	410.72284	-2.62375	0.77133	-3.40158	6.6998E-04	2.9134E-03
K11740	412.05599	-2.62282	0.77140	-3.40007	6.7370E-04	2.9134E-03
K03533	412.23903	-2.62282	0.77156	-3.39935	6.7546E-04	2.9134E-03
K07310	412.23903	-2.62282	0.77156	-3.39935	6.7546E-04	2.9134E-03
K02567	412.41589	-2.62158	0.77171	-3.39712	6.8099E-04	2.9134E-03
K02570	412.41589	-2.62158	0.77171	-3.39712	6.8099E-04	2.9134E-03
K03620	412.43839	-2.62083	0.77130	-3.39793	6.7898E-04	2.9134E-03
K04065	412.26429	-2.61999	0.76843	-3.40954	6.5074E-04	2.9134E-03
K03855	410.82084	-2.61949	0.77146	-3.39552	6.8498E-04	2.9134E-03
K05796	413.73135	-2.61784	0.76670	-3.41444	6.3913E-04	2.9134E-03
K07312	413.91439	-2.61784	0.76686	-3.41372	6.4083E-04	2.9134E-03
K08298	413.91439	-2.61784	0.76686	-3.41372	6.4083E-04	2.9134E-03
K19222	413.91439	-2.61784	0.76686	-3.41372	6.4083E-04	2.9134E-03
K03646	414.18039	-2.61661	0.76565	-3.41751	6.3196E-04	2.9134E-03
K12297	414.05923	-2.61661	0.76698	-3.41160	6.4583E-04	2.9134E-03
K01974	409.33445	-2.61260	0.78085	-3.34584	8.2032E-04	3.0900E-03
K11936	411.84509	-2.61085	0.77162	-3.38360	7.1543E-04	2.9134E-03
K03748	411.50897	-2.61012	0.76911	-3.39370	6.8954E-04	2.9134E-03
K02858	410.97779	-2.60765	0.77620	-3.35950	7.8083E-04	2.9591E-03
K02427	411.15465	-2.60642	0.77634	-3.35734	7.8697E-04	2.9721E-03
K06176	411.15465	-2.60642	0.77634	-3.35734	7.8697E-04	2.9721E-03
K14187	411.15465	-2.60642	0.77634	-3.35734	7.8697E-04	2.9721E-03
K00127	414.49103	-2.60576	0.76748	-3.39519	6.8580E-04	2.9134E-03
K07306	418.99541	-2.60311	0.75906	-3.42939	6.0494E-04	2.9134E-03
K03721	413.50388	-2.59359	0.75988	-3.41316	6.4213E-04	2.9134E-03
K07309	418.46883	-2.58409	0.75591	-3.41852	6.2963E-04	2.9134E-03
K12136	419.92913	-2.57934	0.75083	-3.43533	5.9184E-04	2.9134E-03
K11904	2485.81513	-2.57454	0.78035	-3.29923	9.6951E-04	3.5961E-03

DEGs d 42: NC + 500 FTU/kg vs. NC (1,340 DEGs)

GeneID	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj
K02380	419.73430	-2.54969	0.74486	-3.42306	6.1921E-04	2.9134E-03
K02362	425.27578	-2.54555	0.72454	-3.51333	4.4252E-04	2.9134E-03
K14054	425.27578	-2.54555	0.72454	-3.51333	4.4252E-04	2.9134E-03
K02680	427.06396	-2.53980	0.72211	-3.51720	4.3613E-04	2.9134E-03
K06867	2079.08452	-2.52271	0.77277	-3.26451	1.0965E-03	4.0026E-03
K00568	451.81679	-2.52235	0.72137	-3.49662	4.7120E-04	2.9134E-03
K07722	419.05637	-2.52220	0.75854	-3.32508	8.8393E-04	3.3096E-03
K03649	422.31488	-2.51872	0.75164	-3.35095	8.0534E-04	3.0362E-03
K08314	423.91293	-2.51550	0.74616	-3.37128	7.4821E-04	2.9134E-03
K01577	419.81447	-2.49934	0.73761	-3.38843	7.0293E-04	2.9134E-03
K01595	421.11602	-2.48187	0.73544	-3.37466	7.3907E-04	2.9134E-03
K02573	436.57666	-2.47380	0.72500	-3.41214	6.4454E-04	2.9134E-03
K02574	436.75969	-2.47380	0.72516	-3.41141	6.4628E-04	2.9134E-03
K07787	435.67017	-2.43298	0.70622	-3.44505	5.7094E-04	2.9134E-03
K07798	435.67017	-2.43298	0.70622	-3.44505	5.7094E-04	2.9134E-03
K00684	438.67735	-2.42878	0.70292	-3.45528	5.4972E-04	2.9134E-03
K02393	437.77335	-2.40486	0.70456	-3.41326	6.4190E-04	2.9134E-03
K02394	437.77335	-2.40486	0.70456	-3.41326	6.4190E-04	2.9134E-03
K03841	437.95022	-2.40381	0.70468	-3.41120	6.4677E-04	2.9134E-03
K02193	441.59118	-2.40114	0.69126	-3.47355	5.1362E-04	2.9134E-03
K02194	441.53506	-2.40080	0.69123	-3.47321	5.1427E-04	2.9134E-03
K02195	441.53506	-2.40080	0.69123	-3.47321	5.1427E-04	2.9134E-03
K02197	441.53506	-2.40080	0.69123	-3.47321	5.1427E-04	2.9134E-03
K02198	441.53506	-2.40080	0.69123	-3.47321	5.1427E-04	2.9134E-03
K00758	434.22765	-2.38196	0.70812	-3.36377	7.6884E-04	2.9228E-03
K03224	434.05035	-2.38163	0.70912	-3.35855	7.8353E-04	2.9668E-03
K11179	434.19520	-2.38061	0.70922	-3.35664	7.8897E-04	2.9771E-03
K01070	860.20025	-2.34134	0.70332	-3.32900	8.7158E-04	3.2690E-03
K11249	446.58213	-2.33687	0.69353	-3.36951	7.5301E-04	2.9134E-03
K11931	446.58213	-2.33687	0.69353	-3.36951	7.5301E-04	2.9134E-03
K07008	437.92445	-2.33629	0.70327	-3.32202	8.9367E-04	3.3404E-03
K11183	446.75899	-2.33591	0.69366	-3.36749	7.5855E-04	2.9134E-03
K01920	431.08381	-2.31208	0.69336	-3.33459	8.5426E-04	3.2096E-03
K11076	442.51752	-2.31077	0.68214	-3.38753	7.0525E-04	2.9134E-03
K05982	440.53169	-2.30862	0.69736	-3.31050	9.3130E-04	3.4691E-03
K07308	475.37311	-2.30084	0.67660	-3.40056	6.7247E-04	2.9134E-03
K02854	435.55724	-2.29664	0.70192	-3.27192	1.0682E-03	3.9123E-03
K12660	435.55724	-2.29664	0.70192	-3.27192	1.0682E-03	3.9123E-03
K13918	435.55724	-2.29664	0.70192	-3.27192	1.0682E-03	3.9123E-03

DEGs d 42: NC + 500 FTU/kg vs. NC (1,340 DEGs)

GeneID	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj
K15922	435.55724	-2.29664	0.70192	-3.27192	1.0682E-03	3.9123E-03
K04343	437.20058	-2.29320	0.70151	-3.26896	1.0795E-03	3.9448E-03
K10984	435.93373	-2.29272	0.70137	-3.26890	1.0797E-03	3.9448E-03
K02532	878.21322	-2.29096	0.70003	-3.27265	1.0654E-03	3.9123E-03
K01069	438.41248	-2.26836	0.70110	-3.23545	1.2145E-03	4.4038E-03
K03684	438.47665	-2.25684	0.67916	-3.32299	8.9059E-04	3.3317E-03
K01085	516.25616	-2.25680	0.65396	-3.45097	5.5858E-04	2.9134E-03
K07214	1312.64327	-2.25199	0.68012	-3.31115	9.2913E-04	3.4640E-03
K09913	450.18812	-2.22024	0.70276	-3.15932	1.5814E-03	5.6634E-03
K06952	917.93586	-2.21988	0.64397	-3.44721	5.6642E-04	2.9134E-03
K05844	450.61640	-2.21830	0.70226	-3.15881	1.5842E-03	5.6687E-03
K03782	466.16753	-2.18438	0.65782	-3.32063	8.9814E-04	3.3513E-03
K19416	466.16753	-2.18438	0.65782	-3.32063	8.9814E-04	3.3513E-03
K00325	460.81280	-2.17972	0.64208	-3.39476	6.8689E-04	2.9134E-03
K05801	458.53340	-2.17296	0.69752	-3.11528	1.8377E-03	6.5437E-03
K00230	471.13818	-2.13531	0.66033	-3.23371	1.2219E-03	4.4233E-03
K02478	940.11378	-2.13276	0.60521	-3.52397	4.2514E-04	2.9134E-03
K17464	465.82975	-2.13229	0.63214	-3.37315	7.4314E-04	2.9134E-03
K02048	915.97119	-2.11051	0.64564	-3.26886	1.0798E-03	3.9448E-03
K00216	452.56418	-2.09177	0.69481	-3.01054	2.6078E-03	9.1149E-03
K01252	452.56418	-2.09177	0.69481	-3.01054	2.6078E-03	9.1149E-03
K02363	452.56418	-2.09177	0.69481	-3.01054	2.6078E-03	9.1149E-03
K11103	452.56418	-2.09177	0.69481	-3.01054	2.6078E-03	9.1149E-03
K03638	453.31587	-2.09176	0.69576	-3.00646	2.6431E-03	9.1820E-03
K04091	452.44302	-2.09176	0.69712	-3.00056	2.6948E-03	9.2923E-03
K11735	452.44302	-2.09176	0.69712	-3.00056	2.6948E-03	9.2923E-03
K16263	452.44302	-2.09176	0.69712	-3.00056	2.6948E-03	9.2923E-03
K10680	452.61988	-2.09094	0.69724	-2.99887	2.7098E-03	9.3293E-03
K07250	452.80671	-2.09060	0.69267	-3.01817	2.5431E-03	8.9459E-03
K03417	452.68555	-2.09060	0.69478	-3.00900	2.6211E-03	9.1537E-03
K03761	452.56438	-2.09060	0.69709	-2.99903	2.7084E-03	9.3293E-03
K01720	452.80296	-2.08961	0.69474	-3.00777	2.6318E-03	9.1820E-03
K01638	454.20752	-2.08918	0.69471	-3.00726	2.6362E-03	9.1820E-03
K02300	454.95921	-2.08918	0.69565	-3.00320	2.6715E-03	9.2412E-03
K03893	454.95921	-2.08918	0.69565	-3.00320	2.6715E-03	9.2412E-03
K00090	454.08635	-2.08918	0.69702	-2.99729	2.7240E-03	9.3411E-03
K02170	454.08635	-2.08918	0.69702	-2.99729	2.7240E-03	9.3411E-03
K05816	454.08635	-2.08918	0.69702	-2.99729	2.7240E-03	9.3411E-03
K08989	452.77051	-2.08879	0.69688	-2.99736	2.7233E-03	9.3411E-03

DEGs d 42: NC + 500 FTU/kg vs. NC (1,340 DEGs)

GeneID	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj
K06988	453.54240	-2.08803	0.69191	-3.01777	2.5464E-03	8.9504E-03
K01637	454.32889	-2.08802	0.69468	-3.00573	2.6494E-03	9.1939E-03
K01669	455.08057	-2.08802	0.69562	-3.00168	2.6849E-03	9.2802E-03
K00285	454.20772	-2.08802	0.69699	-2.99576	2.7376E-03	9.3805E-03
K00116	456.21944	-2.08720	0.69220	-3.01534	2.5669E-03	9.0153E-03
K02553	454.47373	-2.08720	0.69477	-3.00418	2.6630E-03	9.2264E-03
K14393	454.47373	-2.08720	0.69477	-3.00418	2.6630E-03	9.2264E-03
K07113	454.35256	-2.08720	0.69708	-2.99421	2.7515E-03	9.4208E-03
K03837	454.50199	-2.08621	0.69706	-2.99286	2.7638E-03	9.4553E-03
K07300	455.97398	-2.08257	0.68619	-3.03499	2.4054E-03	8.4959E-03
K03923	456.08097	-2.07869	0.69587	-2.98719	2.8156E-03	9.6176E-03
K08151	459.17131	-2.07862	0.68608	-3.02973	2.4478E-03	8.6315E-03
K14392	460.33599	-2.06567	0.68191	-3.02923	2.4518E-03	8.6387E-03
K09136	457.60494	-2.05529	0.68201	-3.01357	2.5819E-03	9.0532E-03
K03762	467.55291	-2.04078	0.66875	-3.05163	2.2760E-03	8.0716E-03
K02806	499.03354	-2.03583	0.62436	-3.26065	1.1116E-03	4.0473E-03
K08296	499.03354	-2.03583	0.62436	-3.26065	1.1116E-03	4.0473E-03
K05788	499.43702	-2.03372	0.62422	-3.25800	1.1220E-03	4.0820E-03
K03926	500.39752	-2.03188	0.62268	-3.26313	1.1019E-03	4.0188E-03
K07757	470.45599	-2.01987	0.69687	-2.89851	3.7494E-03	1.2639E-02
K00881	465.00059	-2.01322	0.66949	-3.00709	2.6376E-03	9.1820E-03
K01608	465.00059	-2.01322	0.66949	-3.00709	2.6376E-03	9.1820E-03
K02046	464.13974	-2.01180	0.67955	-2.96049	3.0715E-03	1.0467E-02
K02047	464.13974	-2.01180	0.67955	-2.96049	3.0715E-03	1.0467E-02
K00573	502.31936	-2.00613	0.62029	-3.23420	1.2198E-03	4.4193E-03
K12507	471.12572	-1.99087	0.65494	-3.03979	2.3674E-03	8.3753E-03
K08317	483.94016	-1.97158	0.69161	-2.85070	4.3623E-03	1.4637E-02
K10778	485.86108	-1.96689	0.69014	-2.84999	4.3720E-03	1.4659E-02
K00813	489.30484	-1.96175	0.59731	-3.28433	1.0223E-03	3.7706E-03
K10708	565.15002	-1.95871	0.56213	-3.48446	4.9313E-04	2.9134E-03
K13889	565.15002	-1.95871	0.56213	-3.48446	4.9313E-04	2.9134E-03
K19784	486.45413	-1.95857	0.68231	-2.87050	4.0982E-03	1.3794E-02
K03449	1085.22339	-1.95349	0.57878	-3.37520	7.3763E-04	2.9134E-03
K06281	1072.41007	-1.95072	0.55418	-3.51999	4.3157E-04	2.9134E-03
K06282	1070.94754	-1.94955	0.55493	-3.51316	4.4280E-04	2.9134E-03
K03556	563.29804	-1.94849	0.56741	-3.43399	5.9476E-04	2.9134E-03
K00164	484.53152	-1.94537	0.60959	-3.19128	1.4164E-03	5.0977E-03
K13628	484.20921	-1.94491	0.61063	-3.18509	1.4471E-03	5.1952E-03
K08310	483.33635	-1.94491	0.61243	-3.17573	1.4946E-03	5.3614E-03

DEGs d 42: NC + 500 FTU/kg vs. NC (1,340 DEGs)

GeneID	**baseMean**	**log2FoldChange**	**lfcSE**	**stat**	**pvalue**	**padj**
K03605	1074.22423	-1.93595	0.55479	-3.48950	4.8392E-04	2.9134E-03
K02472	526.33458	-1.91765	0.60916	-3.14801	1.6439E-03	5.8727E-03
K00529	457.46780	-1.91333	0.72159	-2.65153	8.0127E-03	2.5700E-02
K02043	457.46780	-1.91333	0.72159	-2.65153	8.0127E-03	2.5700E-02
K09020	457.46780	-1.91333	0.72159	-2.65153	8.0127E-03	2.5700E-02
K10537	457.46780	-1.91333	0.72159	-2.65153	8.0127E-03	2.5700E-02
K10544	457.46780	-1.91333	0.72159	-2.65153	8.0127E-03	2.5700E-02
K17463	457.46780	-1.91333	0.72159	-2.65153	8.0127E-03	2.5700E-02
K17468	457.46780	-1.91333	0.72159	-2.65153	8.0127E-03	2.5700E-02
K06145	459.28800	-1.91056	0.72267	-2.64375	8.1992E-03	2.6240E-02
K06165	527.82658	-1.90186	0.59595	-3.19131	1.4163E-03	5.0977E-03
K11924	527.82658	-1.90186	0.59595	-3.19131	1.4163E-03	5.0977E-03
K06188	479.65626	-1.86884	0.63588	-2.93898	3.2929E-03	1.1169E-02
K00324	513.89002	-1.84515	0.58733	-3.14162	1.6802E-03	5.9975E-03
K04085	486.49531	-1.83134	0.65426	-2.79911	5.1244E-03	1.6959E-02
K16370	468.89480	-1.78919	0.71258	-2.51088	1.2043E-02	3.7657E-02
K00795	470.71500	-1.78688	0.71367	-2.50379	1.2287E-02	3.8283E-02
K05916	515.17403	-1.78331	0.60307	-2.95707	3.1058E-03	1.0576E-02
K07034	513.45133	-1.77031	0.60235	-2.93903	3.2924E-03	1.1169E-02
K00694	1243.90721	-1.77009	0.53610	-3.30178	9.6072E-04	3.5696E-03
K10036	514.15809	-1.76634	0.62124	-2.84324	4.4658E-03	1.4927E-02
K17722	514.15809	-1.76634	0.62124	-2.84324	4.4658E-03	1.4927E-02
K00299	648.26856	-1.74464	0.57231	-3.04840	2.3006E-03	8.1521E-03
K13497	516.76404	-1.74282	0.61789	-2.82058	4.7936E-03	1.5937E-02
K07400	525.14916	-1.71565	0.60091	-2.85507	4.3027E-03	1.4448E-02
K03611	500.59234	-1.71198	0.66455	-2.57615	9.9906E-03	3.1556E-02
K00383	524.98122	-1.70806	0.60988	-2.80067	5.0997E-03	1.6890E-02
K17467	530.31379	-1.70804	0.60642	-2.81660	4.8535E-03	1.6112E-02
K10550	486.91114	-1.69996	0.67827	-2.50632	1.2200E-02	3.8038E-02
K08996	659.11692	-1.69579	0.56265	-3.01391	2.5791E-03	9.0506E-03
K03616	549.20776	-1.69074	0.52853	-3.19897	1.3792E-03	4.9802E-03
K13953	509.90516	-1.68109	0.61914	-2.71519	6.6237E-03	2.1636E-02
K01176	1197.99946	-1.66361	0.55614	-2.99135	2.7775E-03	9.4948E-03
K06999	531.44017	-1.63950	0.62575	-2.62007	8.7911E-03	2.7950E-02
K07146	531.67894	-1.63818	0.62557	-2.61869	8.8269E-03	2.8043E-02
K02173	624.30035	-1.63790	0.54479	-3.00648	2.6429E-03	9.1820E-03
K07120	539.62531	-1.62916	0.57476	-2.83452	4.5895E-03	1.5305E-02
K02784	570.10224	-1.62549	0.61473	-2.64422	8.1879E-03	2.6223E-02
K02549	539.49114	-1.62408	0.61132	-2.65669	7.8913E-03	2.5460E-02

DEGs d 42: NC + 500 FTU/kg vs. NC (1,340 DEGs)

GeneID	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj
K03779	538.15424	-1.61939	0.58311	-2.77714	5.4840E-03	1.8067E-02
K07223	677.83734	-1.61372	0.54738	-2.94806	3.1978E-03	1.0880E-02
K00073	555.68482	-1.59129	0.58876	-2.70277	6.8765E-03	2.2384E-02
K01066	1096.61230	-1.55707	0.55011	-2.83047	4.6480E-03	1.5489E-02
K19540	552.74684	-1.54843	0.58093	-2.66545	7.6885E-03	2.4843E-02
K10551	1163.41142	-1.53613	0.50453	-3.04467	2.3294E-03	8.2474E-03
K02552	568.36777	-1.53470	0.54296	-2.82652	4.7057E-03	1.5669E-02
K08680	568.24660	-1.53470	0.54348	-2.82382	4.7455E-03	1.5789E-02
K10549	523.31477	-1.53210	0.63351	-2.41843	1.5588E-02	4.7448E-02
K15866	552.02985	-1.52206	0.56226	-2.70702	6.7889E-03	2.2116E-02
K01096	695.06392	-1.51991	0.55094	-2.75874	5.8025E-03	1.9059E-02
K00557	697.65690	-1.51644	0.54929	-2.76071	5.7677E-03	1.8959E-02
K03636	542.08147	-1.51407	0.60690	-2.49477	1.2604E-02	3.9213E-02
K02441	566.59597	-1.50158	0.52535	-2.85823	4.2602E-03	1.4317E-02
K03272	581.05681	-1.48401	0.59691	-2.48614	1.2914E-02	4.0093E-02
K09927	1252.39744	-1.46725	0.50174	-2.92429	3.4524E-03	1.1692E-02
K06193	597.67172	-1.45001	0.55732	-2.60176	9.2746E-03	2.9358E-02
K08368	720.43829	-1.44763	0.53144	-2.72397	6.4502E-03	2.1091E-02
K01664	575.98923	-1.44487	0.53276	-2.71203	6.6872E-03	2.1817E-02
K14977	583.48482	-1.44068	0.56858	-2.53383	1.1282E-02	3.5405E-02
K11203	1674.32455	-1.43154	0.51748	-2.76637	5.6684E-03	1.8647E-02
K03767	594.07119	-1.42089	0.56995	-2.49303	1.2666E-02	3.9350E-02
K07160	740.86612	-1.41707	0.43766	-3.23779	1.2046E-03	4.3714E-03
K10010	722.30649	-1.41619	0.52521	-2.69642	7.0089E-03	2.2748E-02
K11733	726.74065	-1.41109	0.52476	-2.68903	7.1660E-03	2.3206E-02
K00124	716.02094	-1.40556	0.46753	-3.00637	2.6439E-03	9.1820E-03
K06917	721.15635	-1.40431	0.43966	-3.19408	1.4028E-03	5.0610E-03
K01185	4489.91773	-1.39703	0.54839	-2.54752	1.0849E-02	3.4120E-02
K17466	597.56772	-1.38966	0.54667	-2.54208	1.1020E-02	3.4606E-02
K06191	756.85578	-1.37561	0.51076	-2.69328	7.0754E-03	2.2929E-02
K00598	753.21531	-1.34095	0.44320	-3.02560	2.4814E-03	8.7359E-03
K00832	790.17478	-1.33414	0.48227	-2.76640	5.6679E-03	1.8647E-02
K02791	964.81475	-1.32313	0.47176	-2.80465	5.0371E-03	1.6696E-02
K04087	708.39036	-1.31024	0.45061	-2.90771	3.6409E-03	1.2302E-02
K04088	708.39036	-1.31024	0.45061	-2.90771	3.6409E-03	1.2302E-02
K18148	707.58696	-1.31006	0.45085	-2.90573	3.6640E-03	1.2370E-02
K05786	673.22759	-1.30122	0.46828	-2.77870	5.4576E-03	1.7994E-02
K11202	1820.97215	-1.25241	0.48926	-2.55982	1.0473E-02	3.2983E-02
K11102	771.04619	-1.24134	0.51188	-2.42503	1.5307E-02	4.6655E-02

DEGs d 42: NC + 500 FTU/kg vs. NC (1,340 DEGs)

GeneID	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj
K07180	755.41811	-1.23956	0.44344	-2.79529	5.1853E-03	1.7135E-02
K09786	755.41811	-1.23956	0.44344	-2.79529	5.1853E-03	1.7135E-02
K01582	1319.49571	-1.23573	0.50709	-2.43689	1.4814E-02	4.5283E-02
K03635	706.75831	-1.19827	0.48036	-2.49453	1.2613E-02	3.9213E-02
K02783	1666.30878	-1.18136	0.46448	-2.54341	1.0978E-02	3.4499E-02
K09788	728.09295	-1.16590	0.43850	-2.65887	7.8403E-03	2.5314E-02
K03931	794.91422	-1.13750	0.46518	-2.44528	1.4474E-02	4.4430E-02
K10545	757.29752	-1.07653	0.42912	-2.50870	1.2118E-02	3.7836E-02
K15553	799.58617	-1.05116	0.40901	-2.57001	1.0170E-02	3.2098E-02
K07497	9285.24273	-1.03241	0.30581	-3.37595	7.3560E-04	2.9134E-03
K02302	976.11030	-1.01475	0.36970	-2.74480	6.0548E-03	1.9843E-02
K06426	339.56808	1.01633	0.41331	2.45900	1.3932E-02	4.2919E-02
K18940	289.84602	1.01948	0.42164	2.41787	1.5611E-02	4.7488E-02
K16149	49.64273	1.16817	0.44105	2.64859	8.0827E-03	2.5905E-02
K00966	217.33485	1.23938	0.45718	2.71090	6.7101E-03	2.1876E-02
K10819	52.88304	1.25028	0.43949	2.84486	4.4431E-03	1.4874E-02
K19081	38.94857	1.64627	0.51607	3.19001	1.4227E-03	5.1118E-03
K19082	38.94857	1.64627	0.51607	3.19001	1.4227E-03	5.1118E-03

Number of up- and down-regulated differentially expressed genes (DEGs) based on predicted metagenome of the cecal microbiota compared between YPM x Ross 708 male broilers provided a negative control (NC; 0 FTU/kg) diet and a NC with phytase supplementation (500 or 1,500 FTU/kg) on d 28 and 42.

DEGs d 42: NC + 1,500 FTU/kg vs. NC (4 DEGs)						
GeneID	**baseMean**	**log2FoldChange**	**lfcSE**	**stat**	**pvalue**	**padj**
K05524	875.06977	-1.47084	0.32095	-4.58281	4.5877E-06	3.4860E-03
K02496	1283.00386	-1.31357	0.37709	-3.48348	4.9493E-04	4.6285E-02
K09680	1313.13294	-1.09910	0.24360	-4.51183	6.4269E-06	3.4860E-03
K17950	816.00184	-1.04748	0.29091	-3.60070	3.1736E-04	3.4427E-02

Number of up- and down-regulated differentially expressed genes (DEGs) based on predicted metagenome of the cecal microbiota in YPM x Ross 708 male broilers provided a negative control diet varying in phytase, calcium butyrate (CB), and bacitracin methylene disalicylate (BMD) inclusion on d 28 and 42.

DEGs d 28: 1,500 FTU/kg vs. 500 FTU/kg (4 DEGs)						
GeneID	**baseMean**	**log2FoldChange**	**lfcSE**	**stat**	**pvalue**	**padj**
K00897	13.37507	-20.22903	2.07524	-9.74781	1.8800E-22	2.4800E-19
K12268	13.37507	-20.22903	2.07524	-9.74781	1.8800E-22	2.4800E-19
K12269	13.37507	-20.22903	2.07524	-9.74781	1.8800E-22	2.4800E-19
K12270	13.37507	-20.22903	2.07524	-9.74781	1.8800E-22	2.4800E-19

Number of up- and down-regulated differentially expressed genes (DEGs) based on predicted metagenome of the cecal microbiota in YPM x Ross 708 male broilers provided a negative control diet varying in phytase, calcium butyrate (CB), and bacitracin methylene disalicylate (BMD) inclusion on d 28 and 42.

DEGs d 28: All other MMFA vs. None (3 DEGs)						
GeneID	**baseMean**	**log2FoldChange**	**lfcSE**	**stat**	**pvalue**	**padj**
K13727	6.22663	21.26985	4.76689	4.46200	8.1198E-06	1.4229E-02
K17869	6.22663	21.26985	4.76689	4.46200	8.1198E-06	1.4229E-02
K06216	12.45325	22.41266	4.76663	4.70199	2.5764E-06	1.3544E-02

Number of up- and down-regulated differentially expressed genes (DEGs) based on predicted metagenome of the cecal microbiota in YPM x Ross 708 male broilers provided a negative control diet varying in phytase, calcium butyrate (CB), and bacitracin methylene disalicylate (BMD) inclusion on d 28 and 42.

DEGs d 28: Only BMD vs. None (7 DEGs)						
GeneID	**baseMean**	**log2FoldChange**	**lfcSE**	**stat**	**pvalue**	**padj**
K05568	6186.32278	-1.11773	0.25554	-4.37394	1.2203E-05	1.3642E-02
K05571	2062.19280	-1.11768	0.26138	-4.27610	1.9020E-05	1.3642E-02
K05567	2062.20645	-1.11768	0.26137	-4.27617	1.9013E-05	1.3642E-02
K05569	2062.20645	-1.11768	0.26137	-4.27617	1.9013E-05	1.3642E-02
K05570	2062.20645	-1.11768	0.26137	-4.27617	1.9013E-05	1.3642E-02
K05566	2062.25539	-1.11768	0.26135	-4.27655	1.8981E-05	1.3642E-02
K06216	12.45325	24.98081	5.81570	4.29541	1.7437E-05	1.3642E-02

Number of up- and down-regulated differentially expressed genes (DEGs) based on predicted metagenome of the cecal microbiota in YPM x Ross 708 male broilers provided a negative control diet varying in phytase, calcium butyrate (CB), and bacitracin methylene disalicylate (BMD) inclusion on d 28 and 42.

DEGs d 28: Both CB and BMD vs. None (4 DEGs)						
GeneID	**baseMean**	**log2FoldChange**	**lfcSE**	**stat**	**pvalue**	**padj**
K00897	13.37507	-16.45124	2.10168	-7.82764	4.9711E-15	6.5357E-12
K12268	13.37507	-16.45124	2.10168	-7.82764	4.9711E-15	6.5357E-12
K12269	13.37507	-16.45124	2.10168	-7.82764	4.9711E-15	6.5357E-12
K12270	13.37507	-16.45124	2.10168	-7.82764	4.9711E-15	6.5357E-12

Number of up- and down-regulated differentially expressed genes (DEGs) based on predicted metagenome of the cecal microbiota in YPM x Ross 708 male broilers provided a negative control diet varying in phytase, calcium butyrate (CB), and bacitracin methylene disalicylate (BMD) inclusion on d 28 and 42.

DEGs d 42: Only BMD vs. None (1 DEG)						
GeneID	**baseMean**	**log2FoldChange**	**lfcSE**	**stat**	**pvalue**	**padj**
K16093	7.08703	-38.03683	5.84654	-6.50587	7.7244E-11	3.9441E-07

Number of up- and down-regulated differentially expressed genes (DEGs) based on predicted metagenome of the cecal microbiota in YPM x Ross 708 male broilers provided a negative control diet varying in phytase, calcium butyrate (CB), and bacitracin methylene disalicylate (BMD) inclusion on d 28 and 42.

DEGs d 42: Both CB and BMD vs. None (43 DEGs)

GeneID	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj
K02347	506.43150	-2.00970	0.54930	-3.65869	2.5351E-04	4.9023E-03
K02275	440.04413	-1.37143	0.51767	-2.64922	8.0679E-03	4.3783E-02
K06285	1093.13908	-1.07722	0.33726	-3.19403	1.4030E-03	1.5349E-02
K01206	16316.93643	-1.04378	0.29560	-3.53101	4.1397E-04	6.5331E-03
K08999	4308.79637	-1.01614	0.31760	-3.19941	1.3771E-03	1.5126E-02
K01802	8315.22945	-1.00710	0.32151	-3.13240	1.7338E-03	1.7681E-02
K08676	4090.58643	-1.00701	0.33210	-3.03226	2.4273E-03	2.2292E-02
K01966	4123.10784	-1.00296	0.33014	-3.03796	2.3818E-03	2.1948E-02
K03735	5595.73703	1.00836	0.28150	3.58212	3.4082E-04	5.8852E-03
K03736	5601.14938	1.01186	0.28190	3.58942	3.3142E-04	5.8338E-03
K03825	3153.58075	1.02704	0.33740	3.04400	2.3345E-03	2.1803E-02
K01640	3064.13025	1.04049	0.28147	3.69666	2.1846E-04	4.4341E-03
K16203	3045.72449	1.04646	0.27568	3.79587	1.4713E-04	3.3950E-03
K17206	1501.20329	1.05198	0.30922	3.40208	6.6876E-04	8.8715E-03
K01029	2024.64466	1.06602	0.31446	3.39000	6.9893E-04	9.2272E-03
K18122	1496.83782	1.06837	0.28428	3.75815	1.7117E-04	3.6722E-03
K02106	3215.45730	1.08364	0.28994	3.73743	1.8591E-04	3.9270E-03
K03457	1779.21629	1.09898	0.29845	3.68229	2.3115E-04	4.5995E-03
K17899	1372.23479	1.10805	0.29426	3.76558	1.6616E-04	3.6213E-03
K01844	1469.79882	1.11010	0.29055	3.82070	1.3307E-04	3.2432E-03
K18011	1469.79882	1.11010	0.29055	3.82070	1.3307E-04	3.2432E-03
K02230	1718.16604	1.11069	0.33088	3.35679	7.8853E-04	1.0059E-02
K01476	1391.02636	1.11848	0.30993	3.60877	3.0765E-04	5.4504E-03
K17898	1377.66310	1.12160	0.29568	3.79331	1.4865E-04	3.4016E-03
K13919	1428.50260	1.14216	0.29965	3.81163	1.3805E-04	3.2749E-03
K13920	1428.50260	1.14216	0.29965	3.81163	1.3805E-04	3.2749E-03
K06859	1336.67534	1.14525	0.30462	3.75954	1.7023E-04	3.6722E-03
K18012	2747.11000	1.15429	0.29428	3.92237	8.7683E-05	2.5615E-03
K01699	1433.80891	1.15534	0.30110	3.83712	1.2449E-04	3.1652E-03
K18118	2667.24258	1.16548	0.30397	3.83425	1.2595E-04	3.1730E-03
K18120	6002.91550	1.17159	0.29107	4.02513	5.6945E-05	1.8397E-03
K03405	1276.44326	1.17589	0.30873	3.80877	1.3966E-04	3.2779E-03
K03404	1276.32312	1.17666	0.30875	3.81111	1.3834E-04	3.2749E-03
K16875	1266.45017	1.17681	0.31043	3.79088	1.5012E-04	3.4068E-03
K11618	1421.93213	1.19106	0.29627	4.02020	5.8150E-05	1.8567E-03

DEGs d 42: Both CB and BMD vs. None (43 DEGs)						
GeneID	**baseMean**	**log2FoldChange**	**lfcSE**	**stat**	**pvalue**	**padj**
K17742	1271.86572	1.19129	0.31186	3.81999	1.3346E-04	3.2432E-03
K17818	1271.86572	1.19129	0.31186	3.81999	1.3346E-04	3.2432E-03
K14654	958.07786	1.20786	0.29066	4.15552	3.2455E-05	1.3925E-03
K01028	865.70417	1.23473	0.29764	4.14842	3.3477E-05	1.3929E-03
K17735	2104.07900	1.24399	0.29497	4.21736	2.4718E-05	1.2803E-03
K07654	832.35162	1.32162	0.30537	4.32794	1.5051E-05	8.9846E-04
K11528	832.21328	1.32324	0.30554	4.33088	1.4852E-05	8.9846E-04
K05524	853.58741	1.35978	0.29616	4.59136	4.4037E-06	6.1389E-04

APPENDIX 3

PREDICTED UP- AND DOWN-REGULATED METABOLIC PATHWAYS IN THE CECAL MICROBIOME BETWEEN YPM X ROSS 708 MALE BROILERS PROVIDED A NEGATIVE CONTROL DIET AND A NC WITH PHYTASE SUPPLEMENTATION ON D 28 AND 42

Predicted up- and down-regulated metabolic pathways in the cecal microbiome between YPM x Ross 708 male broilers provided a negative control (NC; 0 FTU/kg) diet and a NC with phytase supplementation (500 or 1,500 FTU/kg) on d 28 and 42.

Downregulated pathways d 28: NC + 500 FTU/kg vs. NC

Category	**Subcategory**	**ID**	**Description**	**Gene Ratio**	**Bg Ratio**	**pvalue**	**p.adjust**	**qvalue**	**GeneID**	**Count**
Metabolism	Amino acid metabolism	ko00260	Glycine, serine and threonine metabolism	0.10	109/14222	1.86E-07	2.54E-06	1.50E-06	K00003/K00018/K00058/K00060/K00090/K00108/K00130/K00133	8
Metabolism	Amino acid metabolism	ko00270	Cysteine and methionine metabolism	0.06	127/14222	7.82E-04	3.37E-03	1.99E-03	K00003/K00016/K00024/K00058/K00133	5
Metabolism	Amino acid metabolism	ko00350	Tyrosine metabolism	0.05	84/14222	1.33E-03	5.21E-03	3.07E-03	K00055/K00121/K00146/K00151	4
Metabolism	Amino acid metabolism	ko00280	Valine, leucine and isoleucine degradation	0.04	71/14222	7.70E-03	2.43E-02	1.43E-02	K00020/K00128/K00140	3
Metabolism	Amino acid metabolism	ko00360	Phenylalanine metabolism	0.04	74/14222	8.63E-03	2.62E-02	1.55E-02	K00055/K00074/K00146	3
Human Diseases	Cancer: overview	ko05230	Central carbon metabolism in cancer	0.05	49/14222	1.70E-04	9.97E-04	5.89E-04	K00016/K00031/K00036/K00161	4
Metabolism	Carbohydrate metabolism	ko00620	Pyruvate metabolism	0.16	133/14222	5.04E-13	2.07E-11	1.22E-11	K00002/K00016/K00024/K00027/K00090/K00101/K00114/K00116/K00121/K00128/K00132/K00156/K00161	13
Metabolism	Carbohydrate metabolism	ko00630	Glyoxylate and dicarboxylate metabolism	0.12	110/14222	6.01E-10	1.64E-08	9.69E-09	K00015/K00018/K00023/K00024/K00042/K00048/K00090/K00104/K00122/K00123	10
Metabolism	Carbohydrate metabolism	ko00010	Glycolysis / Gluconeogenesis	0.11	107/14222	9.13E-09	1.76E-07	1.04E-07	K00002/K00016/K00114/K00121/K00128/K00131/K00134/K00150/K00161	9

Downregulated pathways d 28: NC + 500 FTU/kg vs. NC

Category	**Subcategory**	**ID**	**Description**	**Gene Ratio**	**Bg Ratio**	**pvalue**	**p.adjust**	**qvalue**	**GeneID**	**Count**
Metabolism	Carbohydrate metabolism	ko00030	Pentose phosphate pathway	0.09	89/14222	7.07E-07	7.82E-06	4.62E-06	K00032/K00033/K00034/K00036/K00090/K00117/K00131	7
Metabolism	Carbohydrate metabolism	ko00040	Pentose and glucuronate interconversions	0.09	90/14222	7.63E-07	7.82E-06	4.62E-06	K00002/K00008/K00012/K00040/K00041/K00065/K00078	7
Metabolism	Carbohydrate metabolism	ko00020	Citrate cycle (TCA cycle)	0.06	70/14222	4.79E-05	3.62E-04	2.14E-04	K00024/K00030/K00031/K00116/K00161	5
Metabolism	Carbohydrate metabolism	ko00051	Fructose and mannose metabolism	0.07	115/14222	4.86E-05	3.62E-04	2.14E-04	K00008/K00009/K00045/K00064/K00066/K00068	6
Metabolism	Carbohydrate metabolism	ko00640	Propanoate metabolism	0.06	102/14222	2.86E-04	1.56E-03	9.23E-04	K00005/K00016/K00048/K00086/K00140	5
Metabolism	Carbohydrate metabolism	ko00053	Ascorbate and aldarate metabolism	0.05	62/14222	4.24E-04	2.04E-03	1.21E-03	K00002/K00012/K00064/K00128	4
Metabolism	Carbohydrate metabolism	ko00650	Butanoate metabolism	0.05	114/14222	4.06E-03	1.39E-02	8.18E-03	K00023/K00074/K00100/K00132	4
Metabolism	Energy metabolism	ko00680	Methane metabolism	0.09	195/14222	1.21E-04	7.62E-04	4.50E-04	K00018/K00024/K00058/K00121/K00122/K00123/K00148	7
Metabolism	Energy metabolism	ko00710	Carbon fixation by Calvin cycle	0.04	36/14222	1.11E-03	4.55E-03	2.69E-03	K00024/K00134/K00150	3
Metabolism	Global and overview maps	ko01200	Carbon metabolism	0.26	376/14222	1.35E-15	1.11E-13	6.55E-14	K00018/K00023/K00024/K00027/K00030/K00031/K00033/K00034/K00036/K00058/K00074/K00116/K00121/K00122/K00123/K00131/K00134/K00140/K00148/K00150/K00161	21

Downregulated pathways d 28: NC + 500 FTU/kg vs. NC

Category	**Subcategory**	**ID**	**Description**	**Gene Ratio**	**Bg Ratio**	**pvalue**	**p.adjust**	**qvalue**	**GeneID**	**Count**
Metabolism	Global and overview maps	ko01230	Biosynthesis of amino acids	0.15	241/14222	1.07E-08	1.76E-07	1.04E-07	K00003/K00013/K00014/K00030/K00031/K00052/K00058/K00133/K00134/K00145/K00147/K00150	12
Metabolism	Global and overview maps	ko01210	2-Oxocarboxylic acid metabolism	0.07	113/14222	4.40E-05	3.62E-04	2.14E-04	K00030/K00031/K00052/K00133/K00145/K00161	6
Metabolism	Lipid metabolism	ko00564	Glycerophospholipid metabolism	0.06	117/14222	5.38E-04	2.45E-03	1.45E-03	K00057/K00105/K00111/K00112/K00113	5
Metabolism	Lipid metabolism	ko00561	Glycerolipid metabolism	0.05	94/14222	2.02E-03	7.53E-03	4.44E-03	K00002/K00005/K00086/K00128	4
Metabolism	Metabolism of other amino acids	ko00480	Glutathione metabolism	0.05	57/14222	3.07E-04	1.57E-03	9.28E-04	K00031/K00032/K00033/K00036	4
Metabolism	Metabolism of other amino acids	ko00410	beta-Alanine metabolism	0.04	46/14222	2.26E-03	8.07E-03	4.77E-03	K00128/K00137/K00140	3
Metabolism	Metabolism of terpenoids and polyketides	ko00900	Terpenoid backbone biosynthesis	0.04	63/14222	5.53E-03	1.81E-02	1.07E-02	K00021/K00054/K00099	3
Environmental Information Processing	Signal transduction	ko04066	HIF-1 signaling pathway	0.04	77/14222	9.62E-03	2.82E-02	1.66E-02	K00016/K00134/K00161	3
Metabolism	Xenobiotics biodegradation and metabolism	ko00625	Chloroalkane and chloroalkene degradation	0.05	43/14222	1.02E-04	6.95E-04	4.11E-04	K00114/K00121/K00128/K00148	4

Downregulated pathways d 28: NC + 500 FTU/kg vs. NC

Category	Subcategory	ID	Description	Gene Ratio	Bg Ratio	pvalue	p.adjust	qvalue	GeneID	Count
Metabolism	Xenobiotics biodegradation and metabolism	ko00980	Metabolism of xenobiotics by cytochrome P450	0.02	34/14222	1.60E-02	4.52E-02	2.67E-02	K00078/K00121	2

Predicted up- and down-regulated metabolic pathways in the cecal microbiome between YPM x Ross 708 male broilers provided a negative control (NC; 0 FTU/kg) diet and a NC with phytase supplementation (500 or 1,500 FTU/kg) on d 28 and 42.

Upregulated pathways d 28: NC + 500 FTU/kg vs. NC

Category	**Subcategory**	**ID**	**Description**	**Gene Ratio**	**Bg Ratio**	**pvalue**	**p.adjust**	**qvalue**	**GeneID**	**Count**
Metabolism	Amino acid metabolism	ko00350	Tyrosine metabolism	0.13	84/14222	3.92E-03	2.43E-02	1.24E-02	K00001/K00135	2
Metabolism	Carbohydrate metabolism	ko00620	Pyruvate metabolism	0.31	133/14222	2.67E-07	8.27E-06	4.21E-06	K00001/K00029/K00102/K00138/K00158	5
Metabolism	Carbohydrate metabolism	ko00650	Butanoate metabolism	0.19	114/14222	2.60E-04	4.04E-03	2.06E-03	K00004/K00019/K00135	3
Metabolism	Carbohydrate metabolism	ko00010	Glycolysis / Gluconeogenesis	0.13	107/14222	6.28E-03	2.94E-02	1.49E-02	K00001/K00138	2
Metabolism	Carbohydrate metabolism	ko00630	Glyoxylate and dicarboxylate metabolism	0.13	110/14222	6.63E-03	2.94E-02	1.49E-02	K00124/K00127	2
Metabolism	Energy metabolism	ko00680	Methane metabolism	0.19	195/14222	1.25E-03	9.66E-03	4.92E-03	K00124/K00125/K00127	3
Metabolism	Global and overview maps	ko01200	Carbon metabolism	0.25	376/14222	6.80E-04	7.03E-03	3.58E-03	K00029/K00124/K00125/K00127	4

Predicted up- and down-regulated metabolic pathways in the cecal microbiome between YPM x Ross 708 male broilers provided a negative control (NC; 0 FTU/kg) diet and a NC with phytase supplementation (500 or 1,500 FTU/kg) on d 28 and 42.

Downregulated pathways d 28: NC + 1,500 FTU/kg vs. NC

Category	**Subcategory**	**ID**	**Description**	**Gene Ratio**	**Bg Ratio**	**pvalue**	**p.adjust**	**qvalue**	**GeneID**	**Count**
Metabolism	Amino acid metabolism	ko00280	Valine, leucine and isoleucine degradation	0.09	71/14222	9.50E-08	8.65E-07	4.55E-07	K00020/K00128/K00166/K00167/K00248/K00249/K00253	7
Metabolism	Amino acid metabolism	ko00350	Tyrosine metabolism	0.07	84/14222	8.47E-05	4.63E-04	2.44E-04	K00055/K00121/K00135/K00146/K00151	5
Metabolism	Amino acid metabolism	ko00260	Glycine, serine and threonine metabolism	0.07	109/14222	2.89E-04	1.39E-03	7.34E-04	K00003/K00018/K00058/K00108/K00282	5
Metabolism	Amino acid metabolism	ko00270	Cysteine and methionine metabolism	0.05	127/14222	4.74E-03	1.55E-02	8.18E-03	K00003/K00016/K00024/K00058	4
Metabolism	Amino acid metabolism	ko00360	Phenylalanine metabolism	0.04	74/14222	7.24E-03	2.05E-02	1.08E-02	K00055/K00074/K00146	3
Metabolism	Amino acid metabolism	ko00310	Lysine degradation	0.04	98/14222	1.55E-02	3.81E-02	2.00E-02	K00128/K00135/K00137	3
Human Diseases	Cancer: overview	ko05230	Central carbon metabolism in cancer	0.07	49/14222	6.05E-06	3.82E-05	2.01E-05	K00016/K00031/K00036/K00161/K00162	5
Metabolism	Carbohydrate metabolism	ko00620	Pyruvate metabolism	0.18	133/14222	8.19E-15	3.36E-13	1.77E-13	K00002/K00016/K00024/K00027/K00029/K00114/K00121/K00128/K00158/K00161/K00162/K00171/K00175/K00247	14
Metabolism	Carbohydrate metabolism	ko00020	Citrate cycle (TCA cycle)	0.14	70/14222	8.05E-14	2.20E-12	1.16E-12	K00024/K00030/K00031/K00161/K00162/K00171/K00175/K00176/K00177/K00240/K00247	11

Downregulated pathways d 28: NC + 1,500 FTU/kg vs. NC

Category	Subcategory	ID	Description	Gene Ratio	Bg Ratio	pvalue	p.adjust	qvalue	GeneID	Count
Metabolism	Carbohydrate metabolism	ko00630	Glyoxylate and dicarboxylate metabolism	0.13	110/14222	3.15E-10	5.17E-09	2.72E-09	K00015/K00018/K00023/K00024/K00042/K00104/K00122/K00123/K00127/K00282	10
Metabolism	Carbohydrate metabolism	ko00010	Glycolysis / Gluconeogenesis	0.12	107/14222	5.14E-09	7.03E-08	3.70E-08	K00002/K00016/K00114/K00121/K00128/K00161/K00162/K00171/K00175	9
Metabolism	Carbohydrate metabolism	ko00650	Butanoate metabolism	0.12	114/14222	9.03E-09	1.06E-07	5.57E-08	K00019/K00023/K00074/K00135/K00171/K00175/K00240/K00247/K00248	9
Metabolism	Carbohydrate metabolism	ko00640	Propanoate metabolism	0.09	102/14222	1.16E-06	8.62E-06	4.53E-06	K00005/K00016/K00086/K00166/K00167/K00171/K00248	7
Metabolism	Carbohydrate metabolism	ko00053	Ascorbate and aldarate metabolism	0.04	62/14222	4.42E-03	1.51E-02	7.95E-03	K00002/K00012/K00128	3
Metabolism	Carbohydrate metabolism	ko00040	Pentose and glucuronate interconversions	0.04	90/14222	1.24E-02	3.17E-02	1.67E-02	K00002/K00012/K00065	3
Human Diseases	Endocrine and metabolic disease	ko04936	Alcoholic liver disease	0.04	105/14222	1.86E-02	4.36E-02	2.30E-02	K00121/K00128/K00249	3
Organismal Systems	Endocrine system	ko04922	Glucagon signaling pathway	0.04	57/14222	3.49E-03	1.32E-02	6.94E-03	K00016/K00161/K00162	3
Metabolism	Energy metabolism	ko00720	Other carbon fixation pathways	0.12	121/14222	1.53E-08	1.57E-07	8.24E-08	K00024/K00031/K00171/K00175/K00176/K00177/K00240/K00247/K00297	9
Metabolism	Energy metabolism	ko00680	Methane metabolism	0.11	195/14222	9.19E-06	5.38E-05	2.83E-05	K00018/K00024/K00058/K00121/K00122/K00123/K00127/K00171	8

Downregulated pathways d 28: NC + 1,500 FTU/kg vs. NC

Category	**Subcategory**	**ID**	**Description**	**Gene Ratio**	**Bg Ratio**	**pvalue**	**p.adjust**	**qvalue**	**GeneID**	**Count**
Metabolism	Energy metabolism	ko00710	Carbon fixation by Calvin cycle	0.03	36/14222	1.58E-02	3.81E-02	2.00E-02	K00024/K00029	2
Metabolism	Global and overview maps	ko01200	Carbon metabolism	0.33	376/14222	3.73E-21	3.06E-19	1.61E-19	K00018/K00023/K00024/K00027/K00029/K00030/K00031/K00036/K00058/K00074/K00121/K00122/K00123/K00127/K00161/K00162/K00171/K00175/K00176/K00177/K00240/K00247/K00248/K00282/K00297	25
Metabolism	Global and overview maps	ko01210	2-Oxocarboxylic acid metabolism	0.14	113/14222	1.80E-11	3.70E-10	1.95E-10	K00030/K00031/K00145/K00161/K00162/K00166/K00167/K00171/K00175/K00176/K00177	11
Metabolism	Global and overview maps	ko01230	Biosynthesis of amino acids	0.12	241/14222	5.26E-06	3.60E-05	1.89E-05	K00003/K00013/K00014/K00030/K00031/K00058/K00145/K00215/K00220	9
Metabolism	Global and overview maps	ko01240	Biosynthesis of cofactors	0.09	375/14222	3.84E-03	1.37E-02	7.21E-03	K00002/K00012/K00059/K00082/K00128/K00228/K00230	7
Metabolism	Global and overview maps	ko01220	Degradation of aromatic compounds	0.07	215/14222	5.84E-03	1.77E-02	9.33E-03	K00002/K00055/K00121/K00151/K00217	5
Metabolism	Global and overview maps	ko01212	Fatty acid metabolism	0.04	84/14222	1.03E-02	2.71E-02	1.43E-02	K00059/K00248/K00249	3
Metabolism	Lipid metabolism	ko00071	Fatty acid degradation	0.05	59/14222	2.74E-04	1.39E-03	7.34E-04	K00121/K00128/K00248/K00249	4
Metabolism	Lipid metabolism	ko00561	Glycerolipid metabolism	0.05	94/14222	1.60E-03	6.89E-03	3.63E-03	K00002/K00005/K00086/K00128	4

Downregulated pathways d 28: NC + 1,500 FTU/kg vs. NC

Category	**Subcategory**	**ID**	**Description**	**Gene Ratio**	**Bg Ratio**	**pvalue**	**p.adjust**	**qvalue**	**GeneID**	**Count**
Metabolism	Lipid metabolism	ko00564	Glycerophospholipid metabolism	0.05	117/14222	3.54E-03	1.32E-02	6.94E-03	K00057/K00105/K00112/K00113	4
Metabolism	Metabolism of cofactors and vitamins	ko00785	Lipoic acid metabolism	0.07	31/14222	5.81E-07	4.77E-06	2.51E-06	K00161/K00162/K00166/K00167/K00282	5
Metabolism	Metabolism of cofactors and vitamins	ko00670	One carbon pool by folate	0.04	65/14222	5.05E-03	1.59E-02	8.38E-03	K00108/K00282/K00297	3
Metabolism	Metabolism of cofactors and vitamins	ko00860	Porphyrin metabolism	0.05	142/14222	7.01E-03	2.05E-02	1.08E-02	K00214/K00218/K00228/K00230	4
Metabolism	Metabolism of other amino acids	ko00410	beta-Alanine metabolism	0.04	46/14222	1.89E-03	7.74E-03	4.07E-03	K00128/K00137/K00248	3
Environmental Information Processing	Signal transduction	ko04066	HIF-1 signaling pathway	0.04	77/14222	8.08E-03	2.21E-02	1.16E-02	K00016/K00161/K00162	3
Metabolism	Xenobiotics biodegradation and metabolism	ko00625	Chloroalkane and chloroalkene degradation	0.04	43/14222	1.55E-03	6.89E-03	3.63E-03	K00114/K00121/K00128	3

Predicted up- and down-regulated metabolic pathways in the cecal microbiome between YPM x Ross 708 male broilers provided a negative control (NC; 0 FTU/kg) diet and a NC with phytase supplementation (500 or 1,500 FTU/kg) on d 28 and 42.

Upregulated pathways d 28: NC + 1,500 FTU/kg vs. NC

Category	**Subcategory**	**ID**	**Description**	**Gene Ratio**	**Bg Ratio**	**pvalue**	**p.adjust**	**qvalue**	**GeneID**	**Count**
Metabolism	Amino acid metabolism	ko00260	Glycine, serine and threonine metabolism	0.12	109/14222	1.30E-10	2.10E-09	1.26E-09	K00060/K00090/K00130/K00133/K00274/K00276/K00281/K00283/K00301/K00302/K00303	11
Metabolism	Amino acid metabolism	ko00250	Alanine, aspartate and glutamate metabolism	0.07	70/14222	3.75E-07	3.64E-06	2.17E-06	K00259/K00260/K00261/K00262/K00265/K00266/K00278	7
Metabolism	Amino acid metabolism	ko00290	Valine, leucine and isoleucine biosynthesis	0.03	19/14222	2.51E-04	1.43E-03	8.55E-04	K00052/K00053/K00263	3
Metabolism	Amino acid metabolism	ko00360	Phenylalanine metabolism	0.04	74/14222	1.45E-03	6.38E-03	3.81E-03	K00270/K00274/K00276/K00285	4
Metabolism	Amino acid metabolism	ko00350	Tyrosine metabolism	0.04	84/14222	2.31E-03	9.32E-03	5.56E-03	K00001/K00270/K00274/K00276	4
Metabolism	Amino acid metabolism	ko00220	Arginine biosynthesis	0.03	67/14222	9.87E-03	3.19E-02	1.91E-02	K00260/K00261/K00262	3
Metabolism	Amino acid metabolism	ko00280	Valine, leucine and isoleucine degradation	0.03	71/14222	1.16E-02	3.45E-02	2.06E-02	K00140/K00186/K00263	3
Metabolism	Carbohydrate metabolism	ko00620	Pyruvate metabolism	0.17	133/14222	3.37E-16	1.63E-14	9.76E-15	K00001/K00090/K00101/K00102/K00116/K00132/K00138/K00156/K00163/K00169/K00170/K00172/K00174/K00244/K00245/K00246	16

Upregulated pathways d 28: NC + 1,500 FTU/kg vs. NC

Category	**Subcategory**	**ID**	**Description**	**Gene Ratio**	**Bg Ratio**	**pvalue**	**p.adjust**	**qvalue**	**GeneID**	**Count**
Metabolism	Carbohydrate metabolism	ko00020	Citrate cycle (TCA cycle)	0.14	70/14222	6.79E-16	2.20E-14	1.31E-14	K00116/K00163/K00164/K00169/K00170/K00172/K00174/K00239/K00241/K00242/K00244/K00245/K00246	13
Metabolism	Carbohydrate metabolism	ko00650	Butanoate metabolism	0.15	114/14222	2.04E-14	4.94E-13	2.95E-13	K00004/K00100/K00132/K00169/K00170/K00172/K00174/K00209/K00239/K00241/K00242/K00244/K00245/K00246	14
Metabolism	Carbohydrate metabolism	ko00010	Glycolysis / Gluconeogenesis	0.11	107/14222	2.02E-09	2.81E-08	1.67E-08	K00001/K00131/K00134/K00138/K00150/K00163/K00169/K00170/K00172/K00174	10
Metabolism	Carbohydrate metabolism	ko00640	Propanoate metabolism	0.06	102/14222	5.74E-05	3.71E-04	2.21E-04	K00048/K00140/K00169/K00170/K00172/K00232	6
Metabolism	Carbohydrate metabolism	ko00630	Glyoxylate and dicarboxylate metabolism	0.06	110/14222	8.75E-05	5.30E-04	3.17E-04	K00048/K00090/K00124/K00281/K00283/K00284	6
Metabolism	Carbohydrate metabolism	ko00030	Pentose phosphate pathway	0.05	89/14222	3.04E-04	1.64E-03	9.76E-04	K00032/K00033/K00034/K00090/K00131	5
Metabolism	Carbohydrate metabolism	ko00040	Pentose and glucuronate interconversions	0.04	90/14222	2.96E-03	1.15E-02	6.86E-03	K00008/K00040/K00041/K00078	4
Metabolism	Carbohydrate metabolism	ko00051	Fructose and mannose metabolism	0.04	115/14222	7.08E-03	2.45E-02	1.46E-02	K00008/K00009/K00045/K00064	4

Upregulated pathways d 28: NC + 1,500 FTU/kg vs. NC										
Category	**Subcategory**	**ID**	**Description**	**Gene Ratio**	**Bg Ratio**	**pvalue**	**p.adjust**	**qvalue**	**GeneID**	**Count**
Metabolism	Energy metabolism	ko00720	Other carbon fixation pathways	0.15	121/14222	4.75E-14	9.21E-13	5.50E-13	K00169/K00170/K00172/K00174/K00194/K00196/K00197/K00198/K00239/K00241/K00242/K00244/K00245/K00246	14
Metabolism	Energy metabolism	ko00680	Methane metabolism	0.11	195/14222	6.21E-07	5.47E-06	3.27E-06	K00124/K00125/K00148/K00169/K00170/K00172/K00194/K00196/K00197/K00198	10
Metabolism	Energy metabolism	ko00910	Nitrogen metabolism	0.06	69/14222	6.08E-06	4.54E-05	2.71E-05	K00260/K00261/K00262/K00265/K00266/K00284	6
Metabolism	Energy metabolism	ko00190	Oxidative phosphorylation	0.06	224/14222	3.67E-03	1.32E-02	7.88E-03	K00239/K00241/K00242/K00244/K00245/K00246	6
Metabolism	Global and overview maps	ko01200	Carbon metabolism	0.33	376/14222	5.27E-26	5.12E-24	3.05E-24	K00033/K00034/K00116/K00124/K00125/K00131/K00134/K00140/K00148/K00150/K00163/K00164/K00169/K00170/K00172/K00174/K00194/K00196/K00197/K00198/K00209/K00232/K00239/K00241/K00242/K00244/K00245/K00246/K00261/K00281/K00283	31
Metabolism	Global and overview maps	ko01210	2-Oxocarboxylic acid metabolism	0.11	113/14222	3.46E-09	4.20E-08	2.51E-08	K00052/K00053/K00133/K00163/K00164/K00169/K00170/K00172/K00174/K00186	10
Metabolism	Global and overview maps	ko01230	Biosynthesis of amino acids	0.11	241/14222	4.21E-06	3.40E-05	2.03E-05	K00052/K00053/K00133/K00134/K00147/K00150/K00265/K00266/K00286/K00290	10

Upregulated pathways d 28: NC + 1,500 FTU/kg vs. NC										
Category	**Subcategory**	**ID**	**Description**	**Gene Ratio**	**Bg Ratio**	**pvalue**	**p.adjust**	**qvalue**	**GeneID**	**Count**
Metabolism	Global and overview maps	ko01240	Biosynthesis of cofactors	0.10	375/14222	8.27E-04	4.01E-03	2.39E-03	K00077/K00097/K00208/K00226/K00231/K00254/K00275/K00278/K00287	9
Metabolism	Global and overview maps	ko01212	Fatty acid metabolism	0.04	84/14222	2.31E-03	9.32E-03	5.56E-03	K00208/K00209/K00232/K00255	4
Metabolism	Lipid metabolism	ko00071	Fatty acid degradation	0.04	59/14222	6.17E-04	3.15E-03	1.88E-03	K00001/K00232/K00252/K00255	4
Metabolism	Metabolism of cofactors and vitamins	ko00785	Lipoic acid metabolism	0.04	31/14222	4.91E-05	3.40E-04	2.03E-04	K00163/K00164/K00281/K00283	4
Metabolism	Metabolism of cofactors and vitamins	ko00670	One carbon pool by folate	0.04	65/14222	8.90E-04	4.11E-03	2.45E-03	K00130/K00281/K00283/K00287	4
Metabolism	Metabolism of cofactors and vitamins	ko00750	Vitamin B6 metabolism	0.02	24/14222	1.09E-02	3.40E-02	2.03E-02	K00097/K00275	2
Metabolism	Metabolism of other amino acids	ko00410	beta-Alanine metabolism	0.03	46/14222	3.46E-03	1.29E-02	7.69E-03	K00140/K00232/K00276	3
Metabolism	Metabolism of other amino acids	ko00430	Taurine and hypotaurine metabolism	0.02	29/14222	1.56E-02	4.46E-02	2.66E-02	K00259/K00260	2
Metabolism	Metabolism of terpenoids and polyketides	ko00900	Terpenoid backbone biosynthesis	0.03	63/14222	8.34E-03	2.79E-02	1.66E-02	K00021/K00054/K00099	3
Metabolism	Xenobiotics biodegradation and metabolism	ko00633	Nitrotoluene degradation	0.05	23/14222	3.47E-07	3.64E-06	2.17E-06	K00169/K00170/K00172/K00196/K00198	5

Upregulated pathways d 28: NC + 1,500 FTU/kg vs. NC

Category	Subcategory	ID	Description	Gene Ratio	Bg Ratio	pvalue	p.adjust	qvalue	GeneID	Count
Metabolism	Xenobiotics biodegradation and metabolism	ko00982	Drug metabolism - cytochrome P450	0.02	25/14222	1.17E-02	3.45E-02	2.06E-02	K00001/K00274	2

Predicted up- and down-regulated metabolic pathways in the cecal microbiome between YPM x Ross 708 male broilers provided a negative control (NC; 0 FTU/kg) diet and a NC with phytase supplementation (500 or 1,500 FTU/kg) on d 28 and 42.

Downregulated pathways d 42: NC + 500 FTU/kg vs. NC

Category	**Subcategory**	**ID**	**Description**	**Gene Ratio**	**Bg Ratio**	**pvalue**	**p.adjust**	**qvalue**	**GeneID**	**Count**
Metabolism	Amino acid metabolism	ko00260	Glycine, serine and threonine metabolism	0.04	109/14222	7.93E-20	1.67E-18	1.15E-18	K00003/K00018/K00058/K00060/K00090/K00130/K00133/K00274/K00276/K00281/K00282/K00283/K00301/K00302/K00303/K00304/K00305/K00382/K00479/K00600/K00605/K00613/K00639/K00643/K00830/K00831/K00836/K00865/K00872/K00928/K00998/K01079/K01620/K01695/K01696/K01697/K01733/K01752/K01753/K01758/K01834/K02203	42
Metabolism	Amino acid metabolism	ko00250	Alanine, aspartate and glutamate metabolism	0.03	70/14222	4.80E-17	8.06E-16	5.59E-16	K00135/K00259/K00260/K00261/K00262/K00265/K00266/K00278/K00294/K00609/K00610/K00764/K00812/K00813/K00820/K00823/K00830/K01424/K01425/K01580/K01744/K01755/K01756/K01779/K01914/K01915/K01939/K01940/K01953/K01955/K01956	31

Downregulated pathways d 42: NC + 500 FTU/kg vs. NC

Category	**Subcategory**	**ID**	**Description**	**Gene Ratio**	**Bg Ratio**	**pvalue**	**p.adjust**	**qvalue**	**GeneID**	**Count**
Metabolism	Amino acid metabolism	ko00280	Valine, leucine and isoleucine degradation	0.03	71/14222	7.91E-17	1.25E-15	8.64E-16	K00020/K00128/K00140/K00166/K00167/K00186/K00248/K00249/K00253/K00263/K00382/K00626/K00632/K00822/K00826/K01027/K01028/K01029/K01640/K01641/K01692/K01782/K01825/K01847/K01848/K01849/K01907/K01965/K01966/K01968/K01969	31
Metabolism	Amino acid metabolism	ko00270	Cysteine and methionine metabolism	0.04	127/14222	6.69E-14	8.87E-13	6.15E-13	K00003/K00016/K00024/K00058/K00133/K00547/K00548/K00549/K00558/K00640/K00641/K00651/K00772/K00789/K00797/K00812/K00813/K00826/K00831/K00832/K00872/K00899/K00928/K01011/K01243/K01251/K01505/K01611/K01697/K01738/K01739/K01740/K01752/K01758/K01760/K01761/K01919/K01920	38

Downregulated pathways d 42: NC + 500 FTU/kg vs. NC

Category	Subcategory	ID	Description	Gene Ratio	Bg Ratio	pvalue	p.adjust	qvalue	GeneID	Count
Metabolism	Amino acid metabolism	ko00330	Arginine and proline metabolism	0.03	115/14222	3.74E-13	4.10E-12	2.84E-12	K00128/K00137/K00147/K00274/K00286/K00294/K00318/K00472/K00491/K00613/K00657/K00673/K00797/K00812/K00813/K00819/K00824/K00840/K00931/K01259/K01426/K01470/K01473/K01474/K01476/K01480/K01484/K01485/K01581/K01583/K01584/K01585/K01611/K01750/K01777	35
Metabolism	Amino acid metabolism	ko00220	Arginine biosynthesis	0.02	67/14222	7.16E-13	7.51E-12	5.21E-12	K00145/K00260/K00261/K00262/K00491/K00611/K00619/K00620/K00812/K00813/K00818/K00821/K00926/K00930/K01425/K01428/K01429/K01430/K01438/K01476/K01478/K01755/K01915/K01940/K01955/K01956	26
Metabolism	Amino acid metabolism	ko00350	Tyrosine metabolism	0.02	84/14222	7.68E-09	5.23E-08	3.63E-08	K00001/K00055/K00121/K00135/K00146/K00151/K00270/K00274/K00276/K00450/K00451/K00455/K00457/K00483/K00484/K00505/K00812/K00813/K00817/K00832/K01555/K01800/K01801/K01826	24

Downregulated pathways d 42: NC + 500 FTU/kg vs. NC

Category	**Subcategory**	**ID**	**Description**	**Gene Ratio**	**Bg Ratio**	**pvalue**	**p.adjust**	**qvalue**	**GeneID**	**Count**
Metabolism	Amino acid metabolism	ko00290	Valine, leucine and isoleucine biosynthesis	0.01	19/14222	1.92E-08	1.24E-07	8.59E-08	K00052/K00053/K00263/K00826/K00835/K01649/K01652/K01653/K01687/K01703/K01704	11
Metabolism	Amino acid metabolism	ko00340	Histidine metabolism	0.01	47/14222	1.67E-07	1.00E-06	6.94E-07	K00013/K00128/K00274/K00603/K00765/K00817/K01089/K01468/K01479/K01496/K01523/K01590/K01693/K01712/K01745/K01814	16
Metabolism	Amino acid metabolism	ko00400	Phenylalanine, tyrosine and tryptophan biosynthesis	0.02	74/14222	3.68E-07	2.16E-06	1.50E-06	K00014/K00220/K00270/K00500/K00766/K00800/K00812/K00813/K00817/K00832/K00891/K01609/K01626/K01657/K01658/K01695/K01696/K01735/K01736/K01817	20
Metabolism	Amino acid metabolism	ko00360	Phenylalanine metabolism	0.02	74/14222	1.69E-06	8.50E-06	5.89E-06	K00055/K00074/K00146/K00270/K00274/K00276/K00285/K00457/K00500/K00529/K00812/K00813/K00817/K00824/K00832/K01426/K01666/K01692/K01912	19
Metabolism	Amino acid metabolism	ko00300	Lysine biosynthesis	0.01	48/14222	7.86E-06	3.74E-05	2.59E-05	K00003/K00133/K00215/K00290/K00674/K00821/K00841/K00928/K01439/K01586/K01714/K01778/K01928/K01929	14

Downregulated pathways d 42: NC + 500 FTU/kg vs. NC

Category	Subcategory	ID	Description	Gene Ratio	Bg Ratio	pvalue	p.adjust	qvalue	GeneID	Count
Metabolism	Amino acid metabolism	ko00380	Tryptophan metabolism	0.01	80/14222	8.71E-04	3.28E-03	2.27E-03	K00128/K00252/K00274/K00382/K00453/K00466/K00626/K00658/K01426/K01501/K01556/K01667/K01692/K01782/K01825	15
Metabolism	Amino acid metabolism	ko00310	Lysine degradation	0.01	98/14222	2.71E-03	9.48E-03	6.58E-03	K00128/K00135/K00137/K00252/K00290/K00382/K00626/K00658/K00824/K01035/K01582/K01692/K01782/K01825/K01843/K01844	16
Metabolism	Biosynthesis of other secondary metabolites	ko00521	Streptomycin biosynthesis	0.01	21/14222	9.69E-07	4.98E-06	3.46E-06	K00010/K00067/K00844/K00845/K00973/K01092/K01710/K01790/K01835/K01858	10
Metabolism	Biosynthesis of other secondary metabolites	ko00261	Monobactam biosynthesis	0.01	28/14222	8.33E-04	3.28E-03	2.27E-03	K00133/K00215/K00928/K00955/K00956/K00957/K00958/K01714	8
Human Diseases	Cancer: overview	ko05230	Central carbon metabolism in cancer	0.01	49/14222	3.22E-03	1.11E-02	7.71E-03	K00016/K00031/K00036/K00161/K00162/K00844/K00850/K00873/K01425/K01834	10

Downregulated pathways d 42: NC + 500 FTU/kg vs. NC

Category	**Subcategory**	**ID**	**Description**	**Gene Ratio**	**Bg Ratio**	**pvalue**	**p.adjust**	**qvalue**	**GeneID**	**Count**
Metabolism	Carbohydrate metabolism	ko00620	Pyruvate metabolism	0.06	133/14222	1.90E-34	1.20E-32	8.31E-33	K00001/K00002/K00016/K00024/K00027/K00029/K00090/K00101/K00102/K00114/K00116/K00121/K00128/K00132/K00138/K00156/K00158/K00161/K00162/K00163/K00169/K00170/K00171/K00172/K00174/K00175/K00244/K00245/K00246/K00247/K00382/K00625/K00626/K00627/K00656/K00873/K00925/K01006/K01007/K01026/K01069/K01512/K01571/K01573/K01595/K01596/K01610/K01638/K01649/K01676/K01677/K01678/K01679/K01759/K01895/K01958/K01959/K01960/K01961/K01962/K01963/K02160	62

Downregulated pathways d 42: NC + 500 FTU/kg vs. NC

Category	**Subcategory**	**ID**	**Description**	**Gene Ratio**	**Bg Ratio**	**pvalue**	**p.adjust**	**qvalue**	**GeneID**	**Count**
Metabolism	Carbohydrate metabolism	ko00020	Citrate cycle (TCA cycle)	0.04	70/14222	7.24E-31	3.65E-29	2.53E-29	K00024/K00030/K00031/K00116/K00161/K00162/K00163/K00164/K00169/K00170/K00171/K00172/K00174/K00175/K00176/K00177/K00239/K00240/K00241/K00242/K00244/K00245/K00246/K00247/K00382/K00627/K00658/K01596/K01610/K01616/K01647/K01659/K01676/K01677/K01678/K01679/K01681/K01682/K01902/K01903/K01958/K01959/K01960	43
Metabolism	Carbohydrate metabolism	ko00650	Butanoate metabolism	0.05	114/14222	1.82E-25	7.62E-24	5.29E-24	K00004/K00019/K00023/K00074/K00100/K00132/K00135/K00169/K00170/K00171/K00172/K00174/K00175/K00209/K00239/K00240/K00241/K00242/K00244/K00245/K00246/K00247/K00248/K00626/K00634/K00656/K00823/K00929/K01027/K01028/K01029/K01035/K01039/K01040/K01574/K01575/K01580/K01615/K01640/K01641/K01652/K01653/K01692/K01715/K01782/K01799/K01825/K01896/K01907	49

Downregulated pathways d 42: NC + 500 FTU/kg vs. NC

Category	**Subcategory**	**ID**	**Description**	**Gene Ratio**	**Bg Ratio**	**pvalue**	**p.adjust**	**qvalue**	**GeneID**	**Count**
Metabolism	Carbohydrate metabolism	ko00010	Glycolysis / Gluconeogenesis	0.04	107/14222	5.58E-25	1.76E-23	1.22E-23	K00001/K00002/K00016/K00114/K00121/K00128/K00131/K00134/K00138/K00150/K00161/K00162/K00163/K00169/K00170/K00171/K00172/K00174/K00175/K00382/K00627/K00844/K00845/K00850/K00873/K00886/K00895/K00918/K00927/K01006/K01007/K01085/K01222/K01223/K01596/K01610/K01622/K01623/K01624/K01689/K01785/K01792/K01803/K01810/K01834/K01835/K01895	47
Metabolism	Carbohydrate metabolism	ko00630	Glyoxylate and dicarboxylate metabolism	0.04	110/14222	2.43E-24	6.12E-23	4.24E-23	K00015/K00018/K00023/K00024/K00042/K00048/K00090/K00104/K00122/K00123/K00124/K00127/K00281/K00282/K00283/K00284/K00382/K00600/K00605/K00626/K00830/K00865/K01091/K01433/K01455/K01569/K01577/K01601/K01602/K01608/K01616/K01625/K01637/K01638/K01647/K01659/K01681/K01682/K01816/K01846/K01847/K01848/K01849/K01895/K01915/K01965/K01966	47

Downregulated pathways d 42: NC + 500 FTU/kg vs. NC

Category	Subcategory	ID	Description	Gene Ratio	Bg Ratio	pvalue	p.adjust	qvalue	GeneID	Count
Metabolism	Carbohydrate metabolism	ko00640	Propanoate metabolism	0.04	102/14222	4.27E-22	9.78E-21	6.78E-21	K00005/K00016/K00048/K00086/K00140/K00166/K00167/K00169/K00170/K00171/K00172/K00232/K00248/K00382/K00625/K00656/K00822/K00823/K00925/K00932/K01026/K01637/K01659/K01682/K01692/K01699/K01720/K01734/K01782/K01825/K01847/K01848/K01849/K01895/K01902/K01903/K01908/K01961/K01962/K01963/K01965/K01966/K02160	43
Metabolism	Carbohydrate metabolism	ko00051	Fructose and mannose metabolism	0.03	115/14222	6.57E-14	8.87E-13	6.15E-13	K00008/K00009/K00045/K00064/K00066/K00068/K00594/K00844/K00847/K00848/K00850/K00863/K00879/K00881/K00882/K00895/K00966/K00971/K01218/K01622/K01623/K01624/K01628/K01629/K01711/K01729/K01795/K01803/K01805/K01808/K01809/K01813/K01818/K01820/K01840/K02377	36

Downregulated pathways d 42: NC + 500 FTU/kg vs. NC

Category	**Subcategory**	**ID**	**Description**	**Gene Ratio**	**Bg Ratio**	**pvalue**	**p.adjust**	**qvalue**	**GeneID**	**Count**
Metabolism	Carbohydrate metabolism	ko00030	Pentose phosphate pathway	0.03	89/14222	1.41E-13	1.77E-12	1.23E-12	K00032/K00033/K00034/K00036/K00090/K00117/K00131/K00615/K00616/K00850/K00851/K00852/K00874/K00895/K00918/K00948/K01053/K01057/K01619/K01621/K01622/K01623/K01624/K01625/K01690/K01783/K01807/K01808/K01810/K01835/K01839	31
Metabolism	Carbohydrate metabolism	ko00500	Starch and sucrose metabolism	0.03	106/14222	1.47E-13	1.77E-12	1.23E-12	K00688/K00690/K00691/K00692/K00694/K00695/K00697/K00700/K00702/K00703/K00705/K00844/K00845/K00847/K00963/K00975/K00978/K01087/K01176/K01179/K01182/K01187/K01193/K01194/K01200/K01210/K01222/K01223/K01226/K01232/K01236/K01810/K01835/K01838	34

Downregulated pathways d 42: NC + 500 FTU/kg vs. NC

Category	Subcategory	ID	Description	Gene Ratio	Bg Ratio	pvalue	p.adjust	qvalue	GeneID	Count
Metabolism	Carbohydrate metabolism	ko00520	Amino sugar and nucleotide sugar metabolism	0.04	157/14222	2.29E-13	2.63E-12	1.82E-12	K00012/K00066/K00075/K00523/K00790/K00820/K00844/K00845/K00847/K00849/K00884/K00885/K00886/K00963/K00965/K00966/K00971/K00972/K00975/K00978/K00983/K00992/K01183/K01198/K01207/K01209/K01443/K01452/K01639/K01654/K01709/K01711/K01784/K01787/K01788/K01791/K01809/K01810/K01835/K01840/K01854/K02377	42
Metabolism	Carbohydrate metabolism	ko00040	Pentose and glucuronate interconversions	0.03	90/14222	4.74E-11	3.98E-10	2.76E-10	K00002/K00008/K00012/K00040/K00041/K00065/K00078/K00594/K00848/K00853/K00854/K00879/K00880/K00963/K01051/K01195/K01628/K01629/K01685/K01686/K01730/K01783/K01804/K01805/K01812/K01815/K01818/K01820	28
Metabolism	Carbohydrate metabolism	ko00052	Galactose metabolism	0.02	78/14222	1.47E-09	1.06E-08	7.34E-09	K00094/K00844/K00845/K00849/K00850/K00883/K00917/K00963/K00965/K01182/K01187/K01190/K01193/K01220/K01631/K01635/K01684/K01784/K01785/K01819/K01835/K01854/K02079/K02082	24

Downregulated pathways d 42: NC + 500 FTU/kg vs. NC

Category	Subcategory	ID	Description	Gene Ratio	Bg Ratio	pvalue	p.adjust	qvalue	GeneID	Count
Metabolism	Carbohydrate metabolism	ko00660	C5-Branched dibasic acid metabolism	0.01	29/14222	1.98E-04	7.92E-04	5.49E-04	K00052/K01575/K01652/K01653/K01703/K01704/K01846/K01902/K01903	9
Metabolism	Carbohydrate metabolism	ko00053	Ascorbate and aldarate metabolism	0.01	62/14222	2.10E-03	7.45E-03	5.17E-03	K00002/K00012/K00064/K00128/K00880/K01053/K01195/K01630/K01683/K01706/K01707/K01708	12
Cellular Processes	Cell motility	ko02040	Flagellar assembly	0.01	55/14222	9.32E-06	4.27E-05	2.96E-05	K02386/K02387/K02388/K02389/K02390/K02391/K02392/K02393/K02394/K02395/K02396/K02397/K02398/K02399/K02400	15
Metabolism	Energy metabolism	ko00720	Other carbon fixation pathways	0.05	121/14222	5.23E-25	1.76E-23	1.22E-23	K00024/K00031/K00169/K00170/K00171/K00172/K00174/K00175/K00176/K00177/K00194/K00196/K00197/K00198/K00239/K00240/K00241/K00242/K00244/K00245/K00246/K00247/K00297/K00625/K00626/K00925/K01006/K01007/K01491/K01595/K01676/K01677/K01678/K01679/K01681/K01682/K01847/K01848/K01849/K01895/K01902/K01903/K01938/K01958/K01959/K01960/K01961/K01962/K01963/K02160	50

Downregulated pathways d 42: NC + 500 FTU/kg vs. NC

Category	Subcategory	ID	Description	Gene Ratio	Bg Ratio	pvalue	p.adjust	qvalue	GeneID	Count
Metabolism	Energy metabolism	ko00190	Oxidative phosphorylation	0.06	224/14222	2.99E-19	5.80E-18	4.03E-18	K00239/K00240/K00241/K00242/K00244/K00245/K00246/K00247/K00330/K00331/K00332/K00333/K00334/K00335/K00336/K00337/K00338/K00339/K00340/K00341/K00342/K00343/K00404/K00405/K00406/K00407/K00410/K00411/K00412/K00413/K00425/K00426/K00937/K01507/K02107/K02108/K02109/K02110/K02111/K02112/K02113/K02114/K02115/K02117/K02118/K02119/K02120/K02121/K02122/K02123/K02124/K02258/K02259/K02274/K02275/K02276/K02277/K02297/K02298/K02299/K02300	61
Metabolism	Energy metabolism	ko00710	Carbon fixation by Calvin cycle	0.02	36/14222	1.08E-12	1.09E-11	7.57E-12	K00024/K00029/K00134/K00150/K00615/K00616/K00855/K00927/K01006/K01595/K01601/K01602/K01610/K01623/K01624/K01783/K01803/K01807/K01808	19

Downregulated pathways d 42: NC + 500 FTU/kg vs. NC

Category	Subcategory	ID	Description	Gene Ratio	Bg Ratio	pvalue	p.adjust	qvalue	GeneID	Count
Metabolism	Energy metabolism	ko00910	Nitrogen metabolism	0.02	69/14222	1.19E-11	1.15E-10	8.01E-11	K00260/K00261/K00262/K00265/K00266/K00284/K00362/K00363/K00366/K00367/K00368/K00370/K00371/K00372/K00374/K00376/K00459/K00926/K01455/K01501/K01673/K01674/K01725/K01915/K02305	25
Metabolism	Energy metabolism	ko00680	Methane metabolism	0.04	195/14222	1.24E-10	9.75E-10	6.76E-10	K00018/K00024/K00058/K00121/K00122/K00123/K00124/K00125/K00127/K00148/K00169/K00170/K00171/K00172/K00194/K00196/K00197/K00198/K00317/K00399/K00400/K00401/K00402/K00441/K00600/K00625/K00830/K00831/K00850/K00863/K00918/K00925/K01007/K01070/K01079/K01595/K01622/K01623/K01624/K01689/K01834/K01895/K02203	43

Downregulated pathways d 42: NC + 500 FTU/kg vs. NC

Category	Subcategory	ID	Description	Gene Ratio	Bg Ratio	pvalue	p.adjust	qvalue	GeneID	Count
Metabolism	Energy metabolism	ko00920	Sulfur metabolism	0.02	118/14222	5.78E-07	3.16E-06	2.19E-06	K00184/K00185/K00299/K00380/K00381/K00385/K00390/K00392/K00394/K00395/K00640/K00641/K00651/K00860/K00955/K00956/K00957/K00958/K01011/K01082/K01738/K01739/K02045/K02046/K02047/K02048	26
Metabolism	Energy metabolism	ko00196	Photosynthesis - antenna proteins	0.01	42/14222	8.03E-06	3.75E-05	2.60E-05	K02092/K02093/K02094/K02095/K02096/K02097/K02284/K02285/K02286/K02287/K02288/K02289/K02290	13

Downregulated pathways d 42: NC + 500 FTU/kg vs. NC

Category	**Subcategory**	**ID**	**Description**	**Gene Ratio**	**Bg Ratio**	**pvalue**	**p.adjust**	**qvalue**	**GeneID**	**Count**
Metabolism	Global and overview maps	ko01200	Carbon metabolism	0.13	376/14222	1.06E-66	2.67E-64	1.85E-64	K00018/K00023/K00024/K00027/K00029/K00030/K00031/K00033/K00034/K00036/K00058/K00074/K00116/K00121/K00122/K00123/K00124/K00125/K00127/K00131/K00134/K00140/K00148/K00150/K00161/K00162/K00163/K00164/K00169/K00170/K00171/K00172/K00174/K00175/K00176/K00177/K00194/K00196/K00197/K00198/K00209/K00232/K00239/K00240/K00241/K00242/K00244/K00245/K00246/K00247/K00248/K00261/K00281/K00282/K00283/K00297/K00317/K00382/K00399/K00401/K00402/K00600/K00605/K00615/K00616/K00625/K00626/K00627/K00640/K00658/K00830/K00831/K00844/K00845/K00850/K00851/K00855/K00863/K00873/K00874/K00886/K00918/K00925/K00926/K00927/K00948/K01006/K01007/K01053/K01057/K01070/K01079/K01455/K01491/K01595/K01601/K01602/K01610/K01616/K01622/K01623/K01624/K01625/K01637/K01638/K01647/K01659/K01676/K01677/K01678/K01679/K01681/K01682/K01689/K01690/K01715/K01720/K01738/K01752/K01782/K01783/K01803/K01807/K01808/K01810/K01825/K01834/K01846/K01847/K01848/K01849/K01895/K01902/K01903/K01938/K01958/K01959/K01960/K01961/K01962/K01963/K01965/K01966/K02160/K02203	145

Downregulated pathways d 42: NC + 500 FTU/kg vs. NC

Category	Subcategory	ID	Description	Gene Ratio	Bg Ratio	pvalue	p.adjust	qvalue	GeneID	Count
Metabolism	Global and overview maps	ko01230	Biosynthesis of amino acids	0.11	241/14222	7.52E-65	9.48E-63	6.57E-63	K00003/K00013/K00014/K00030/K00031/K00052/K00053/K00058/K00133/K00134/K00145/K00147/K00150/K00215/K00220/K00265/K00266/K00286/K00290/K00500/K00548/K00549/K00600/K00611/K00615/K00616/K00619/K00620/K00640/K00641/K00651/K00674/K00765/K00766/K00789/K00800/K00812/K00813/K00817/K00818/K00821/K00826/K00831/K00832/K00836/K00841/K00850/K00872/K00873/K00891/K00918/K00927/K00928/K00930/K00931/K00948/K01079/K01089/K01243/K01438/K01439/K01476/K01496/K01523/K01586/K01609/K01620/K01622/K01623/K01624/K01626/K01647/K01649/K01652/K01653/K01657/K01658/K01659/K01681/K01682/K01687/K01689/K01693/K01695/K01696/K01697/K01703/K01704/K01714/K01733/K01735/K01736/K01738/K01739/K01750/K01752/K01755/K01758/K01760/K01778/K01783/K01803/K01807/K01808/K01814/K01817/K01834/K01914/K01915/K01940/K01953/K01958/K01959/K01960/K02203	115

Downregulated pathways d 42: NC + 500 FTU/kg vs. NC

Category	Subcategory	ID	Description	Gene Ratio	Bg Ratio	pvalue	p.adjust	qvalue	GeneID	Count
Metabolism	Global and overview maps	ko01240	Biosynthesis of cofactors	0.11	375/14222	4.92E-45	4.13E-43	2.86E-43	K00002/K00012/K00059/K00077/K00082/K00097/K00128/K00208/K00226/K00228/K00230/K00231/K00254/K00275/K00278/K00287/K00355/K00382/K00453/K00457/K00568/K00595/K00600/K00606/K00609/K00610/K00643/K00647/K00652/K00762/K00763/K00767/K00768/K00788/K00789/K00793/K00794/K00796/K00798/K00826/K00831/K00833/K00858/K00859/K00867/K00868/K00878/K00939/K00940/K00941/K00946/K00949/K00950/K00954/K00963/K00966/K00969/K01012/K01053/K01077/K01113/K01195/K01465/K01491/K01495/K01497/K01556/K01579/K01591/K01598/K01599/K01633/K01661/K01664/K01665/K01698/K01719/K01737/K01749/K01756/K01772/K01809/K01845/K01885/K01906/K01911/K01916/K01918/K01919/K01920/K01922/K01935/K01937/K01938/K01939/K01950/K01955/K01956/K02169/K02170/K02188/K02189/K02190/K02191/K02224/K02225/K02226/K02227/K02228/K02229/K02230/K02231/K02232/K02233/K02259/K02302/K02303/K02304/K02318/K02372	120

Downregulated pathways d 42: NC + 500 FTU/kg vs. NC

Category	**Subcategory**	**ID**	**Description**	**Gene Ratio**	**Bg Ratio**	**pvalue**	**p.adjust**	**qvalue**	**GeneID**	**Count**
Metabolism	Global and overview maps	ko01210	2-Oxocarboxylic acid metabolism	0.04	113/14222	1.07E-24	3.00E-23	2.08E-23	K00030/K00031/K00052/K00053/K00133/K00145/K00161/K00162/K00163/K00164/K00166/K00167/K00169/K00170/K00171/K00172/K00174/K00175/K00176/K00177/K00186/K00382/K00619/K00620/K00627/K00658/K00812/K00813/K00818/K00821/K00826/K00830/K00836/K00928/K00930/K01438/K01575/K01616/K01647/K01649/K01652/K01653/K01659/K01681/K01682/K01687/K01703/K01704	48
Metabolism	Global and overview maps	ko01232	Nucleotide metabolism	0.04	123/14222	6.06E-16	8.98E-15	6.23E-15	K00087/K00088/K00364/K00525/K00526/K00527/K00560/K00756/K00757/K00758/K00759/K00760/K00761/K00769/K00856/K00857/K00876/K00892/K00939/K00940/K00942/K00943/K00945/K01081/K01129/K01239/K01241/K01250/K01485/K01486/K01487/K01488/K01489/K01493/K01494/K01520/K01756/K01937/K01939/K01951	40

Downregulated pathways d 42: NC + 500 FTU/kg vs. NC

Category	Subcategory	ID	Description	Gene Ratio	Bg Ratio	pvalue	p.adjust	qvalue	GeneID	Count
Metabolism	Global and overview maps	ko01212	Fatty acid metabolism	0.02	84/14222	1.46E-09	1.06E-08	7.34E-09	K00059/K00208/K00209/K00232/K00248/K00249/K00255/K00507/K00626/K00632/K00645/K00647/K00648/K00667/K01692/K01716/K01782/K01825/K01897/K01961/K01962/K01963/K02160/K02371/K02372	25
Metabolism	Global and overview maps	ko01250	Biosynthesis of nucleotide sugars	0.04	211/14222	4.41E-07	2.49E-06	1.72E-06	K00012/K00066/K00067/K00075/K00523/K00790/K00820/K00844/K00845/K00847/K00849/K00884/K00886/K00963/K00965/K00966/K00971/K00972/K00973/K00975/K00978/K00979/K00983/K00992/K01627/K01654/K01709/K01710/K01711/K01784/K01790/K01791/K01809/K01810/K01835/K01840/K01854/K02377	38
Metabolism	Glycan biosynthesis and metabolism	ko00541	Biosynthesis of various nucleotide sugars	0.02	99/14222	1.38E-04	5.69E-04	3.95E-04	K00012/K00067/K00523/K00963/K00971/K00973/K00978/K00983/K01654/K01709/K01710/K01711/K01784/K01790/K01791/K01809/K01840/K01854/K02377	19
Metabolism	Glycan biosynthesis and metabolism	ko00511	Other glycan degradation	0.01	22/14222	8.68E-04	3.28E-03	2.27E-03	K01186/K01190/K01191/K01192/K01201/K01206/K01444	7

Downregulated pathways d 42: NC + 500 FTU/kg vs. NC

Category	**Subcategory**	**ID**	**Description**	**Gene Ratio**	**Bg Ratio**	**pvalue**	**p.adjust**	**qvalue**	**GeneID**	**Count**
Metabolism	Glycan biosynthesis and metabolism	ko00550	Peptidoglycan biosynthesis	0.010	53/14222	1.77E-03	6.38E-03	4.42E-03	K00075/K00687/K00790/K00806/K00887/K01000/K01921/K01924/K01925/K01928/K01929	11
Metabolism	Glycan biosynthesis and metabolism	ko00531	Glycosaminoglycan degradation	0.00	15/14222	3.90E-03	1.33E-02	9.20E-03	K01135/K01195/K01197/K01205/K01207	5
Metabolism	Lipid metabolism	ko00061	Fatty acid biosynthesis	0.01	39/14222	7.47E-09	5.23E-08	3.63E-08	K00059/K00208/K00209/K00645/K00647/K00648/K00667/K01071/K01716/K01897/K01961/K01962/K01963/K02160/K02371/K02372	16
Metabolism	Lipid metabolism	ko00071	Fatty acid degradation	0.01	59/14222	5.12E-06	2.53E-05	1.75E-05	K00001/K00121/K00128/K00232/K00248/K00249/K00252/K00255/K00496/K00529/K00626/K00632/K01692/K01782/K01825/K01897	16
Metabolism	Lipid metabolism	ko00564	Glycerophospholipid metabolism	0.02	117/14222	1.90E-05	8.41E-05	5.83E-05	K00057/K00096/K00105/K00111/K00112/K00113/K00631/K00655/K00901/K00968/K00980/K00981/K00995/K00998/K01004/K01048/K01058/K01095/K01096/K01114/K01126/K01521/K01613	23

Downregulated pathways d 42: NC + 500 FTU/kg vs. NC

Category	**Subcategory**	**ID**	**Description**	**Gene Ratio**	**Bg Ratio**	**pvalue**	**p.adjust**	**qvalue**	**GeneID**	**Count**
Metabolism	Metabolism of cofactors and vitamins	ko00670	One carbon pool by folate	0.02	65/14222	1.92E-11	1.80E-10	1.25E-10	K00130/K00281/K00282/K00283/K00287/K00297/K00382/K00547/K00548/K00560/K00600/K00602/K00603/K00605/K00789/K01251/K01433/K01491/K01492/K01697/K01746/K01758/K01934/K01938	24
Metabolism	Metabolism of cofactors and vitamins	ko00860	Porphyrin metabolism	0.03	142/14222	6.39E-11	5.20E-10	3.60E-10	K00214/K00218/K00228/K00230/K00231/K00510/K00595/K00643/K00768/K00798/K01195/K01599/K01698/K01719/K01749/K01772/K01845/K01885/K02188/K02189/K02190/K02191/K02224/K02225/K02226/K02227/K02228/K02229/K02230/K02231/K02232/K02233/K02259/K02302/K02303/K02304	36
Metabolism	Metabolism of cofactors and vitamins	ko00770	Pantothenate and CoA biosynthesis	0.02	45/14222	1.40E-09	1.06E-08	7.34E-09	K00053/K00077/K00128/K00606/K00826/K00859/K00867/K00954/K00997/K01464/K01579/K01598/K01652/K01653/K01687/K01918/K01922/K02318	18
Metabolism	Metabolism of cofactors and vitamins	ko00785	Lipoic acid metabolism	0.01	31/14222	1.47E-08	9.72E-08	6.74E-08	K00161/K00162/K00163/K00164/K00166/K00167/K00281/K00282/K00283/K00382/K00605/K00627/K00658/K01616	14

Downregulated pathways d 42: NC + 500 FTU/kg vs. NC

Category	Subcategory	ID	Description	Gene Ratio	Bg Ratio	pvalue	p.adjust	qvalue	GeneID	Count
Metabolism	Metabolism of cofactors and vitamins	ko00780	Biotin metabolism	0.01	24/14222	4.44E-07	2.49E-06	1.72E-06	K00059/K00208/K00647/K00652/K00833/K01012/K01906/K01935/K02169/K02170/K02372	11
Metabolism	Metabolism of cofactors and vitamins	ko00730	Thiamine metabolism	0.01	36/14222	1.15E-03	4.26E-03	2.96E-03	K00788/K00878/K00939/K00941/K00946/K00949/K01077/K01078/K01662	9
Metabolism	Metabolism of cofactors and vitamins	ko00740	Riboflavin metabolism	0.01	54/14222	6.66E-03	2.21E-02	1.53E-02	K00082/K00299/K00484/K00793/K00794/K00878/K01078/K01093/K01497/K01515	10
Metabolism	Metabolism of cofactors and vitamins	ko00790	Folate biosynthesis	0.01	84/14222	1.02E-02	3.30E-02	2.29E-02	K00287/K00500/K00796/K00950/K01077/K01113/K01495/K01497/K01633/K01664/K01665/K01724/K01737	13
Metabolism	Metabolism of cofactors and vitamins	ko00760	Nicotinate and nicotinamide metabolism	0.01	97/14222	1.42E-02	4.54E-02	3.15E-02	K00135/K00278/K00322/K00324/K00325/K00763/K00767/K00858/K00952/K00969/K01081/K01799/K01916/K01950	14
Metabolism	Metabolism of other amino acids	ko00450	Selenocompound metabolism	0.01	36/14222	1.46E-07	9.00E-07	6.24E-07	K00384/K00548/K00549/K00955/K00956/K00957/K00958/K01008/K01042/K01739/K01758/K01760/K01761/K01874	14
Metabolism	Metabolism of other amino acids	ko00480	Glutathione metabolism	0.02	57/14222	5.98E-07	3.21E-06	2.22E-06	K00031/K00032/K00033/K00036/K00383/K00432/K00681/K00797/K00799/K01255/K01256/K01270/K01460/K01469/K01581/K01919/K01920	17

Downregulated pathways d 42: NC + 500 FTU/kg vs. NC

Category	Subcategory	ID	Description	Gene Ratio	Bg Ratio	pvalue	p.adjust	qvalue	GeneID	Count
Metabolism	Metabolism of other amino acids	ko00410	beta-Alanine metabolism	0.01	46/14222	7.66E-07	4.02E-06	2.79E-06	K00128/K00137/K00140/K00232/K00248/K00276/K00822/K00823/K01464/K01579/K01580/K01692/K01782/K01825/K01918	15
Metabolism	Metabolism of other amino acids	ko00470	D-Amino acid metabolism	0.01	55/14222	1.81E-04	7.37E-04	5.11E-04	K00285/K00824/K01425/K01586/K01753/K01775/K01776/K01777/K01778/K01779/K01844/K01921/K01925	13
Metabolism	Metabolism of terpenoids and polyketides	ko00900	Terpenoid backbone biosynthesis	0.01	63/14222	1.28E-05	5.76E-05	4.00E-05	K00021/K00054/K00099/K00626/K00795/K00805/K00806/K00869/K00919/K00938/K00991/K01597/K01641/K01662/K01770/K01823	16
NA	NA	ko01310	Nitrogen cycle	0.01	39/14222	1.04E-04	4.42E-04	3.07E-04	K00362/K00363/K00366/K00367/K00368/K00370/K00371/K00372/K00374/K00376/K02305	11
NA	NA	ko04981	Folate transport and metabolism	0.01	24/14222	7.65E-03	2.51E-02	1.74E-02	K00287/K00547/K00548/K00560/K00600/K01934	6

Downregulated pathways d 42: NC + 500 FTU/kg vs. NC

Category	**Subcategory**	**ID**	**Description**	**Gene Ratio**	**Bg Ratio**	**pvalue**	**p.adjust**	**qvalue**	**GeneID**	**Count**
Metabolism	Nucleotide metabolism	ko00230	Purine metabolism	0.05	223/14222	2.43E-17	4.37E-16	3.03E-16	K00073/K00087/K00088/K00364/K00525/K00526/K00527/K00602/K00759/K00760/K00764/K00769/K00839/K00856/K00860/K00892/K00926/K00939/K00940/K00942/K00948/K00951/K00955/K00956/K00957/K00958/K01081/K01119/K01129/K01139/K01239/K01241/K01428/K01429/K01430/K01466/K01477/K01483/K01486/K01487/K01488/K01492/K01515/K01524/K01525/K01588/K01589/K01756/K01768/K01835/K01839/K01923/K01933/K01939/K01945/K01951/K01952/K02083	58
Metabolism	Nucleotide metabolism	ko00240	Pyrimidine metabolism	0.03	114/14222	4.17E-11	3.63E-10	2.51E-10	K00226/K00254/K00525/K00526/K00527/K00560/K00609/K00610/K00756/K00757/K00758/K00761/K00762/K00857/K00876/K00940/K00943/K00945/K01081/K01119/K01250/K01464/K01465/K01485/K01489/K01493/K01494/K01520/K01591/K01937/K01955/K01956	32

Downregulated pathways d 42: NC + 500 FTU/kg vs. NC

Category	Subcategory	ID	Description	Gene Ratio	Bg Ratio	pvalue	p.adjust	qvalue	GeneID	Count
Genetic Information Processing	Replication and repair	ko03030	DNA replication	0.01	60/14222	1.20E-04	5.04E-04	3.50E-04	K01972/K02314/K02316/K02322/K02323/K02335/K02338/K02339/K02340/K02341/K02342/K02343/K02344/K02345	14
Genetic Information Processing	Replication and repair	ko03430	Mismatch repair	0.01	44/14222	1.37E-03	5.01E-03	3.48E-03	K01141/K01972/K02338/K02339/K02340/K02341/K02342/K02343/K02344/K02345	10
Genetic Information Processing	Translation	ko00970	Aminoacyl-tRNA biosynthesis	0.02	66/14222	2.81E-11	2.53E-10	1.75E-10	K00604/K01042/K01866/K01867/K01868/K01869/K01870/K01872/K01873/K01874/K01875/K01876/K01878/K01879/K01880/K01881/K01883/K01885/K01886/K01887/K01889/K01890/K01892/K01893	24
Metabolism	Xenobiotics biodegradation and metabolism	ko00983	Drug metabolism - other enzymes	0.01	32/14222	2.42E-08	1.53E-07	1.06E-07	K00088/K00569/K00757/K00758/K00760/K00799/K00857/K00876/K00940/K01195/K01464/K01489/K01520/K01951	14
Metabolism	Xenobiotics biodegradation and metabolism	ko00643	Styrene degradation	0.01	25/14222	6.78E-06	3.29E-05	2.28E-05	K00146/K00446/K00451/K01026/K01039/K01040/K01426/K01501/K01555/K01800	10

Downregulated pathways d 42: NC + 500 FTU/kg vs. NC

Category	**Subcategory**	**ID**	**Description**	**Gene Ratio**	**Bg Ratio**	**pvalue**	**p.adjust**	**qvalue**	**GeneID**	**Count**
Metabolism	Xenobiotics biodegradation and metabolism	ko00362	Benzoate degradation	0.02	115/14222	4.44E-05	1.93E-04	1.34E-04	K00074/K00217/K00252/K00446/K00448/K00449/K00481/K00529/K00626/K00632/K01031/K01055/K01075/K01607/K01615/K01666/K01692/K01782/K01821/K01825/K01856/K01857	22
Metabolism	Xenobiotics biodegradation and metabolism	ko00930	Caprolactam degradation	0.01	22/14222	8.68E-04	3.28E-03	2.27E-03	K00002/K00496/K01053/K01453/K01692/K01782/K01825	7
Metabolism	Xenobiotics biodegradation and metabolism	ko00633	Nitrotoluene degradation	0.01	23/14222	6.13E-03	2.06E-02	1.43E-02	K00169/K00170/K00171/K00172/K00196/K00198	6

Predicted up- and down-regulated metabolic pathways in the cecal microbiome between YPM x Ross 708 male broilers provided a negative control (NC; 0 FTU/kg) diet and a NC with phytase supplementation (500 or 1,500 FTU/kg) on d 28 and 42.

Upregulated pathways d 42: NC + 500 FTU/kg vs. NC

Category	**Subcategory**	**ID**	**Description**	**Gene Ratio**	**Bg Ratio**	**pvalue**	**p.adjust**	**qvalue**	**GeneID**	**Count**
Metabolism	Amino acid metabolism	ko00260	Glycine, serine and threonine metabolism	0.50	109/14222	8.61E-06	9.47E-05	9.07E-06	K00108/K01754/K02204	3
Metabolism	Amino acid metabolism	ko00290	Valine, leucine and isoleucine biosynthesis	0.17	19/14222	7.99E-03	2.20E-02	2.10E-03	K01754	1
Metabolism	Amino acid metabolism	ko00310	Lysine degradation	0.17	98/14222	4.06E-02	4.97E-02	4.75E-03	K01034	1
Metabolism	Global and overview maps	ko01230	Biosynthesis of amino acids	0.33	241/14222	4.10E-03	1.50E-02	1.44E-03	K01754/K02204	2
Metabolism	Metabolism of cofactors and vitamins	ko00670	One carbon pool by folate	0.17	65/14222	2.71E-02	4.71E-02	4.51E-03	K00108	1
Genetic Information Processing	Replication and repair	ko03030	DNA replication	0.33	60/14222	2.60E-04	1.43E-03	1.37E-04	K02319/K02337	2
Genetic Information Processing	Replication and repair	ko03430	Mismatch repair	0.17	44/14222	1.84E-02	4.05E-02	3.88E-03	K02337	1
Genetic Information Processing	Replication and repair	ko03440	Homologous recombination	0.17	72/14222	3.00E-02	4.71E-02	4.51E-03	K02337	1
Metabolism	Xenobiotics biodegradation and metabolism	ko00627	Aminobenzoate degradation	0.17	89/14222	3.70E-02	4.97E-02	4.75E-03	K01034	1

Predicted up- and down-regulated metabolic pathways in the cecal microbiome between YPM x Ross 708 male broilers provided a negative control (NC; 0 FTU/kg) diet and a NC with phytase supplementation (500 or 1,500 FTU/kg) on d 28 and 42.

Downregulated pathways d 42: NC + 1,500 FTU/kg vs. NC

Category	**Subcategory**	**ID**	**Description**	**Gene Ratio**	**Bg Ratio**	**pvalue**	**p.adjust**	**qvalue**	**GeneID**	**Count**
Metabolism	Amino acid metabolism	ko00300	Lysine biosynthesis	0.25	48/14222	1.34E-02	2.94E-02	2.95E-03	K00003	1
Metabolism	Amino acid metabolism	ko00350	Tyrosine metabolism	0.25	84/14222	2.34E-02	3.67E-02	3.67E-03	K00001	1
Metabolism	Amino acid metabolism	ko00260	Glycine, serine and threonine metabolism	0.25	109/14222	3.03E-02	3.91E-02	3.92E-03	K00003	1
Metabolism	Amino acid metabolism	ko00270	Cysteine and methionine metabolism	0.25	127/14222	3.52E-02	4.11E-02	4.12E-03	K00003	1
Metabolism	Carbohydrate metabolism	ko00010	Glycolysis / Gluconeogenesis	0.50	107/14222	3.33E-04	5.40E-03	5.42E-04	K00001/K00002	2
Metabolism	Carbohydrate metabolism	ko00620	Pyruvate metabolism	0.50	133/14222	5.14E-04	5.40E-03	5.42E-04	K00001/K00002	2
Metabolism	Carbohydrate metabolism	ko00053	Ascorbate and aldarate metabolism	0.25	62/14222	1.73E-02	3.03E-02	3.04E-03	K00002	1
Metabolism	Carbohydrate metabolism	ko00040	Pentose and glucuronate interconversions	0.25	90/14222	2.51E-02	3.67E-02	3.67E-03	K00002	1
Metabolism	Carbohydrate metabolism	ko00650	Butanoate metabolism	0.25	114/14222	3.17E-02	3.91E-02	3.92E-03	K00004	1
Metabolism	Global and overview maps	ko01220	Degradation of aromatic compounds	0.50	215/14222	1.34E-03	9.36E-03	9.39E-04	K00001/K00002	2

Downregulated pathways d 42: NC + 1,500 FTU/kg vs. NC

Category	**Subcategory**	**ID**	**Description**	**Gene Ratio**	**Bg Ratio**	**pvalue**	**p.adjust**	**qvalue**	**GeneID**	**Count**
Metabolism	Lipid metabolism	ko00071	Fatty acid degradation	0.25	59/14222	1.65E-02	3.03E-02	3.04E-03	K00001	1
Metabolism	Lipid metabolism	ko00561	Glycerolipid metabolism	0.25	94/14222	2.62E-02	3.67E-02	3.67E-03	K00002	1
Metabolism	Metabolism of cofactors and vitamins	ko00830	Retinol metabolism	0.25	50/14222	1.40E-02	2.94E-02	2.95E-03	K00001	1
Metabolism	Xenobiotics biodegradation and metabolism	ko00930	Caprolactam degradation	0.25	22/14222	6.17E-03	2.85E-02	2.85E-03	K00002	1
Metabolism	Xenobiotics biodegradation and metabolism	ko00982	Drug metabolism - cytochrome P450	0.25	25/14222	7.01E-03	2.85E-02	2.85E-03	K00001	1
Metabolism	Xenobiotics biodegradation and metabolism	ko00626	Naphthalene degradation	0.25	29/14222	8.13E-03	2.85E-02	2.85E-03	K00001	1
Metabolism	Xenobiotics biodegradation and metabolism	ko00980	Metabolism of xenobiotics by cytochrome P450	0.25	34/14222	9.53E-03	2.86E-02	2.87E-03	K00001	1
Metabolism	Xenobiotics biodegradation and metabolism	ko00625	Chloroalkane and chloroalkene degradation	0.25	43/14222	1.20E-02	2.94E-02	2.95E-03	K00001	1

APPENDIX 4

SUPPLEMENTARY DATA ON DIGESTIBLE NUTRIENT INTAKE, PERFORMANCE, TIBIA MINERALIZATION, AND NUTRIENT DIGESTIBILITY, INCLUDING ORTHOGONAL POLYNOMIAL CONTRASTS

Table S5.1. Independent orthogonal contrast for digestible nutrient intake (units/bird; DM basis) between unchallenged and challenged YPM x Ross 708 male broilers provided diets varying in limestone particle size (PS) and calcium (Ca) concentration between d 17 to 21 and 27 to 35[1].

	200 µm limestone PS[2] and adequate Ca concentration[3]			
Digestible nutrient intake	Unchallenged	Challenged[4]	CLM[5]	*P*-value
CP, g				
d 17 to 21	77.42	81.17	± 2.20	0.019
d 27 to 35	305	311	± 11	0.471
Fat, g				
d 17 to 21	25.79	17.11	± 0.94	<0.001
d 27 to 35	87.08	91.89	± 4.81	0.163
Ca, g				
d 17 to 21	1.38	1.51	± 0.08	0.021
d 27 to 35	4.10	6.76	± 0.50	<0.001
P[6], g				
d 17 to 21	1.50	1.78	± 0.05	<0.001
d 27 to 35	5.63	6.50	± 0.34	0.001
Calorie, kcal				
d 17 to 21	1,402	1,165	± 34	<0.001
d 27 to 35	5,366	5,490	± 151	0.224

[1] Values are least square means of 10 replicate pens; Statistical significance was considered at $P \leq 0.05$.

[2] Limestone was ground using a 2-pair roller mill (Roskamp Champion Series 900-12, California Pellet Mill Co., Crawfordsville, IN) to achieve a PS of 910 µm (coarse) and 200 µm (fine).

[3] Calcium concentration was a two-step, 0.10 percentage unit reduction from the primary breeder's requirements for dietary Ca for each of the growth phases (Starter: Adequate (0.95 %), Reduced (0.85 %), Low (0.75 %); Grower: Adequate (0.85 %), Reduced (0.75 %), Low (0.65 %); Finisher: Adequate (0.75 %), Reduced (0.65 %), Low (0.55 %).

[4] Broilers were enterically challenged with *Eimeria* spp. and *Clostridium perfringens*.

[5] CLM = 95% confidence limit for the mean.

[6] P = Phosphorus.

Table S5.2. Independent orthogonal contrast for digestible essential and total amino acid intake (units/bird; DM basis) between unchallenged and challenged YPM x Ross 708 male broilers provided diets varying in limestone particle size (PS) and calcium (Ca) concentration between d 17 to 21 and 27 to 35[1].

	200 µm limestone PS[2] and adequate Ca concentration[3]			
Digestible nutrient intake	Unchallenged	Challenged[4]	CLM[5]	*P*-value
Met, g				
d 17 to 21	2.97	2.84	± 0.07	0.004
d 27 to 35	9.43	10.62	± 0.30	<0.001
Met + Cys, g				
d 17 to 21	4.05	3.94	± 0.11	0.172
d 27 to 35	13.59	15.02	± 0.50	<0.001
Lys, g				
d 17 to 21	5.48	5.27	± 0.16	0.064
d 27 to 35	20.87	19.60	± 0.83	0.030
Thr, g				
d 17 to 21	3.14	3.00	± 0.12	0.080
d 27 to 35	11.80	11.33	± 0.53	0.184
Val, g				
d 17 to 21	4.20	3.85	± 0.14	0.001
d 27 to 35	15.17	14.22	± 0.61	0.027
Ile, g				
d 17 to 21	3.64	3.48	± 0.12	0.058
d 27 to 35	13.71	12.88	± 0.53	0.026
Arg, g				
d 17 to 21	5.95	5.57	± 0.14	<0.001
d 27 to 35	22.70	21.61	± 0.70	0.026
Trp, g				
d 17 to 21	0.96	1.07	± 0.03	<0.001
d 27 to 35	3.88	3.72	± 0.14	0.104
Leu, g				
d 17 to 21	7.05	6.69	± 0.22	0.020
d 27 to 35	26.62	25.71	± 0.96	0.176
Phe, g				
d 17 to 21	4.24	3.99	± 0.12	0.006
d 27 to 35	15.87	15.42	± 0.57	0.270
His, g				
d 17 to 21	2.30	2.10	± 0.07	<0.001
d 27 to 35	8.42	8.01	± 0.30	0.043
Total AA, g				
d 17 to 21	84.05	79.55	± 2.44	0.011
d 27 to 35	316	303	± 11	0.098

[1] Values are least square means of 10 replicate pens; Statistical significance was considered at $P \leq 0.05$.

[2] Limestone was ground using a 2-pair roller mill (Roskamp Champion Series 900-12, California Pellet Mill Co., Crawfordsville, IN) to achieve a PS of 910 µm (coarse) and 200 µm (fine).

[3] Calcium concentration was a two-step, 0.10 percentage unit reduction from the primary breeder's requirements for dietary Ca for each of the growth phases (Starter: Adequate (0.95 %), Reduced (0.85 %), Low (0.75 %); Grower: Adequate (0.85 %), Reduced (0.75 %), Low (0.65 %); Finisher: Adequate (0.75 %), Reduced (0.65 %), Low (0.55 %).

[4] Broilers were enterically challenged with *Eimeria* spp. and *Clostridium perfringens*.

[5] CLM = 95% confidence limit for the mean.

Table S5.3. Independent orthogonal contrast for digestible non-essential amino acid intake (units/bird; DM basis) between unchallenged and challenged YPM x Ross 708 male broilers provided diets varying in limestone particle size (PS) and calcium (Ca) concentration between d 17 to 21 and 27 to 35[1].

	200 µm limestone PS[2] and adequate Ca concentration[3]			
Digestible nutrient intake	Unchallenged	Challenged[4]	CLM[5]	*P*-value
Ala, g				
d 17 to 21	4.13	3.77	± 0.15	0.001
d 27 to 35	15.43	15.01	± 0.59	0.318
Asp, g				
d 17 to 21	8.29	7.86	± 0.24	0.014
d 27 to 35	32.03	30.45	± 1.12	0.041
Cys, g				
d 17 to 21	1.09	1.10	± 0.04	0.751
d 27 to 35	4.16	4.40	± 0.24	0.139
Glu, g				
d 17 to 21	16.35	15.28	± 0.37	<0.001
d 27 to 35	60.94	59.30	± 1.84	0.191
Gly, g				
d 17 to 21	3.18	3.02	± 0.11	0.040
d 27 to 35	12.37	11.48	± 0.51	0.014
Pro, g				
d 17 to 21	4.90	4.45	± 0.15	<0.001
d 27 to 35	17.71	17.44	± 0.54	0.481
Ser, g				
d 17 to 21	3.42	3.35	± 0.10	0.302
d 27 to 35	13.73	13.58	± 0.48	0.645
Tyr, g				
d 17 to 21	2.72	2.49	± 0.08	<0.001
d 27 to 35	10.47	10.17	± 0.39	0.286

[1] Values are least square means of 10 replicate pens; Statistical significance was considered at $P \leq 0.05$.

[2] Limestone was ground using a 2-pair roller mill (Roskamp Champion Series 900-12, California Pellet Mill Co., Crawfordsville, IN) to achieve a PS of 910 µm (coarse) and 200 µm (fine).

[3] Calcium concentration was a two-step, 0.10 percentage unit reduction from the primary breeder's requirements for dietary Ca for each of the growth phases (Starter: Adequate (0.95 %), Reduced (0.85 %), Low (0.75 %); Grower: Adequate (0.85 %), Reduced (0.75 %), Low (0.65 %); Finisher: Adequate (0.75 %), Reduced (0.65 %), Low (0.55 %).

[4] Broilers were enterically challenged with *Eimeria* spp. and *Clostridium perfringens*.

[5] CLM = 95% confidence limit for the mean.

Table S5.4. Digestible nutrient intake (units/bird; DM basis) of YPM x Ross 708 male broilers provided diets varying in limestone particle size (PS) and calcium (Ca) concentration between d 17 to 21 and 27 to 35[1].

	Main effect									Interaction
	Limestone PS[2], µm (n = 30)				Ca concentration[3], (n = 20)					Limestone PS x Ca concentration
Digestible nutrient intake	910	200	CLM[4]	*P*-value	Adequate	Reduced	Low	CLM	*P*-value	*P*-value
CP, g										
d 17 to 21	76.61	77.46	± 1.36	0.371	79.45	78.07	73.59	± 1.65	<0.001	0.031
d 27 to 35	310	314	± 6	0.299	310^{b}	326^{a}	300^{b}	± 8	<0.001	0.114
Fat, g										
d 17 to 21	17.31	17.13	± 0.61	0.670	16.93	17.76	16.98	± 0.76	0.218	0.615
d 27 to 35	92.92	95.00	± 3.16	0.344	96.41	96.31	89.16	± 3.97	0.011	0.001
Ca, g										
d 17 to 21	1.38	1.37	± 0.04	0.901	1.36	1.43	1.33	± 0.06	0.043	<0.001
d 27 to 35	6.37	5.76	± 0.27	0.001	7.47	5.74	4.99	± 0.34	<0.001	0.005
P[5], g										
d 17 to 21	1.71^{b}	1.80^{a}	± 0.03	<0.001	1.76	1.76	1.74	± 0.04	0.695	0.120
d 27 to 35	6.42	6.67	± 0.19	0.072	6.25^{b}	6.59^{ab}	6.81^{a}	± 0.24	0.005	0.081
Calorie, kcal										
d 17 to 21	1,154	1,152	± 21	0.874	$1,145^{ab}$	$1,186^{a}$	$1,129^{b}$	± 27	0.010	0.126
d 27 to 35	5,496	5,400	± 81	0.091	$5,543^{a}$	$5,616^{a}$	$5,185^{b}$	± 98	<0.001	0.595

[a,b] Means within a row with different superscripts differ significantly ($P \le 0.05$).

[1] Values are least square means of 30 (limestone PS main effect) and 20 (Ca concentration main effect) replicate pens.

[2] Limestone was ground using a 2-pair roller mill (Roskamp Champion Series 900-12, California Pellet Mill Co., Crawfordsville, IN) to achieve a PS of 910 µm (coarse) and 200 µm (fine).

[3] Calcium concentration was a two-step, 0.10 percentage unit reduction from the primary breeder's requirements for dietary Ca for each of the growth phases (Starter: Adequate (0.95 %), Reduced (0.85 %), Low (0.75 %); Grower: Adequate (0.85 %), Reduced (0.75 %), Low (0.65 %); Finisher: Adequate (0.75 %), Reduced (0.65 %), Low (0.55 %).

[4] CLM = 95% confidence limit for the mean.

[5] P = Phosphorus.

Table S5.5. Digestible essential and total amino acid intake (units/bird; DM basis) of YPM x Ross 708 male broilers provided diets varying in limestone particle size (PS) and calcium (Ca) concentration between d 17 to 21 and 27 to 35[1].

	Limestone PS, µm (main effect, n = 30)				Ca concentration[2], (main effect, n = 20)					Limestone PS x Ca concentration
Digestible nutrient intake	910	200	CLM[3]	*P*-value	Adequate	Reduced	Low	CLM	*P*-value	*P*-value
Met, g										
d 17 to 21	2.53	2.58	± 0.04	0.079	2.64	2.55	2.47	± 0.05	<0.001	<0.001
d 27 to 35	10.28	10.88	± 0.16	<0.001	10.25	11.11	10.38	± 0.20	<0.001	<0.001
Met + Cys, g										
d 17 to 21	3.48	3.58	± 0.07	0.042	3.64	3.51	3.44	± 0.09	0.005	<0.001
d 27 to 35	14.60	15.23	± 0.26	0.001	14.78	15.74	14.23	± 0.34	<0.001	0.040
Lys, g										
d 17 to 21	4.81^{b}	5.13^{a}	± 0.10	<0.001	5.16^{a}	4.98^{a}	4.77^{b}	± 0.12	<0.001	0.227
d 27 to 35	20.11	20.47	± 0.47	0.276	19.73	21.37	19.79	± 0.57	<0.001	0.030
Thr, g										
d 17 to 21	2.95	2.93	± 0.07	0.655	3.15	2.85	2.81	± 0.08	<0.001	<0.001
d 27 to 35	11.68	12.13	± 0.29	0.023	11.47	12.26	11.98	± 0.35	0.007	<0.001
Val, g										
d 17 to 21	3.66	3.73	± 0.08	0.220	3.84^{a}	3.68^{ab}	3.55^{b}	± 0.10	0.001	0.274
d 27 to 35	15.06	15.31	± 0.34	0.294	14.70	16.17	14.68	± 0.42	<0.001	0.001
Ile, g										
d 17 to 21	3.21	3.32	± 0.07	0.035	3.42^{a}	3.26^{b}	3.12^{b}	± 0.09	<0.001	0.594
d 27 to 35	13.54	13.81	± 0.30	0.200	13.21	14.65	13.16	± 0.36	<0.001	0.002
Arg, g										
d 17 to 21	5.18	5.47	± 0.08	<0.001	5.50^{a}	5.39^{a}	5.08^{b}	± 0.10	<0.001	0.130
d 27 to 35	22.18	22.50	± 0.39	0.245	21.74	23.76	21.51	± 0.47	<0.001	0.001
Trp, g										
d 17 to 21	0.99	1.04	± 0.02	<0.001	1.07	1.02	0.95	± 0.02	<0.001	<0.001
d 27 to 35	3.72	3.76	± 0.08	0.548	3.75^{ab}	3.83^{a}	3.65^{b}	± 0.10	0.043	0.381
Leu, g										
d 17 to 21	6.36	6.54	± 0.13	0.053	6.60^{a}	6.46^{ab}	6.29^{b}	± 0.17	0.035	0.750
d 27 to 35	26.54	26.71	± 0.54	0.648	26.13^{b}	28.34^{a}	25.40^{b}	± 0.65	<0.001	0.079
Phe, g										
d 17 to 21	3.74^{b}	3.89^{a}	± 0.07	0.007	3.95^{a}	3.80^{ab}	3.69^{b}	± 0.09	0.001	0.260
d 27 to 35	15.74	15.94	± 0.33	0.393	15.53	16.86	15.14	± 0.41	<0.001	0.016
His, g										
d 17 to 21	1.95^{b}	2.04^{a}	± 0.04	0.002	2.07^{a}	2.00^{a}	1.91^{b}	± 0.05	<0.001	0.273
d 27 to 35	8.30	8.40	± 0.16	0.379	8.15	8.88	8.01	± 0.19	<0.001	0.007
Total AA, g										
d 17 to 21	74.52^{b}	77.32^{a}	± 1.50	0.010	78.33^{a}	75.82^{ab}	73.61^{b}	± 1.86	0.003	0.566
d 27 to 35	312	316	± 6	0.379	307	334	301	± 8	<0.001	0.012

^{a,b}Means within a row with different superscripts differ significantly ($P \leq 0.05$). [1] Values are least square means of 30 (limestone PS main effect) and 20 (Ca concentration main effect) replicate pens. [2] Calcium concentration was a two-step, 0.10 percentage unit reduction from the primary breeder's requirements for dietary Ca for each of the growth phases. [3] CLM = 95% confidence limit for the mean.

Table S5.6. Digestible non-essential amino acid intake (units/bird; DM basis) of YPM x Ross 708 male broilers provided diets varying in limestone particle size (PS) and calcium (Ca) concentration between d 17 to 21 and 27 to 35[1].

	Main effect									Interaction
	Limestone PS[2], µm (n = 30)				Ca concentration[3], (n = 20)					Limestone PS x Ca concentration
Digestible nutrient intake	910	200	CLM[4]	*P*-value	Adequate	Reduced	Low	CLM	*P*-value	*P*-value
Ala, g										
d 17 to 21	3.60	3.69	± 0.08	0.149	3.71	3.66	3.57	± 0.10	0.124	0.843
d 27 to 35	15.42	15.40	± 0.34	0.964	15.27^{b}	16.25^{a}	14.71^{b}	± 0.42	<0.001	0.246
Asp, g										
d 17 to 21	7.26^{b}	7.64^{a}	± 0.15	<0.001	7.73^{a}	7.42^{b}	7.20^{b}	± 0.19	0.001	0.169
d 27 to 35	31.22	31.75	± 0.60	0.198	30.70	33.69	30.06	± 0.73	<0.001	<0.001
Cys, g										
d 17 to 21	0.95	1.00	± 0.03	0.002	1.00	0.99	0.93	± 0.03	0.005	<0.001
d 27 to 35	4.25	4.35	± 0.13	0.266	4.35^{b}	4.65^{a}	3.89^{c}	± 0.16	<0.001	0.146
Glu, g										
d 17 to 21	14.29^{b}	14.97^{a}	± 0.22	<0.001	15.04^{a}	14.70^{a}	14.15^{b}	± 0.27	<0.001	0.612
d 27 to 35	60.17	60.98	± 0.96	0.229	59.71	64.48	57.53	± 1.20	<0.001	0.011
Gly, g										
d 17 to 21	2.79^{b}	2.91^{a}	± 0.06	0.011	2.95^{a}	2.86^{a}	2.72^{b}	± 0.08	0.001	0.343
d 27 to 35	12.00	12.06	± 0.29	0.742	11.69	12.79	11.61	± 0.35	<0.001	0.028
Pro, g										
d 17 to 21	4.21^{b}	4.33^{a}	± 0.08	0.047	4.39^{a}	4.29^{ab}	4.14^{b}	± 0.10	0.003	0.714
d 27 to 35	17.31	17.70	± 0.31	0.083	17.51^{b}	18.58^{a}	16.41^{c}	± 0.40	<0.001	0.106
Ser, g										
d 17 to 21	3.03^{b}	3.29^{a}	± 0.06	<0.001	3.21	3.16	3.12	± 0.08	0.319	0.454
d 27 to 35	13.38	13.21	± 0.26	0.348	13.44	14.13	12.32	± 0.32	<0.001	0.001
Tyr, g										
d 17 to 21	2.46	2.55	± 0.05	0.018	2.53	2.55	2.44	± 0.06	0.032	0.010
d 27 to 35	10.27	10.28	± 0.22	0.970	10.21	11.03	9.58	± 0.28	<0.001	0.019

[a-c] Means within a row with different superscripts differ significantly ($P \leq 0.05$).

[1] Values are least square means of 30 (limestone PS main effect) and 20 (Ca concentration main effect) replicate pens.

[2] Limestone was ground using a 2-pair roller mill (Roskamp Champion Series 900-12, California Pellet Mill Co., Crawfordsville, IN) to achieve a PS of 910 µm (coarse) and 200 µm (fine).

[3] Calcium concentration was a two-step, 0.10 percentage unit reduction from the primary breeder's requirements for dietary Ca for each of the growth phases (Starter: Adequate (0.95 %), Reduced (0.85 %), Low (0.75 %); Grower: Adequate (0.85 %), Reduced (0.75 %), Low (0.65 %); Finisher: Adequate (0.75 %), Reduced (0.65 %), Low (0.55 %).

[4] CLM = 95% confidence limit for the mean.

Table S5.7. Performance and mortality responses to calcium (Ca) concentrations as a main effect and within each limestone particle size (PS) group as assessed by orthogonal polynomial contrasts (*P*-values)[1].

	Ca concentration		Limestone particle size[2], µm			
			910		200	
Measurements	Linear	Quadratic	Linear	Quadratic	Linear	Quadratic
BW, g/bird						
d 17	<0.001	0.001	<0.001	0.001	<0.001	0.172
d 21	<0.001	0.003	<0.001	0.002	<0.001	0.279
d 26	<0.001	0.030	<0.001	0.120	<0.001	0.121
d 35	<0.001	<0.001	<0.001	0.004	<0.001	0.001
Feed intake, g/bird						
d 1 to 17	0.003	0.047	0.019	0.129	0.045	0.187
d 17 to 21	0.004	0.066	0.041	0.304	0.029	0.109
d 1 to 21	<0.001	0.024	<0.001	0.024	0.003	0.342
d 18 to 26	<0.001	0.010	0.006	0.080	0.002	0.049
d 1 to 26	<0.001	0.005	0.003	0.045	0.020	0.040
d 27 to 35	<0.001	<0.001	0.119	0.009	<0.001	0.004
d 1 to 35	<0.001	<0.001	<0.001	<0.001	<0.001	0.003
FCR, g:g						
d 1 to 17	<0.001	0.017	<0.001	0.002	<0.001	0.755
d 1 to 21	<0.001	0.138	<0.001	0.106	<0.001	0.632
d 18 to 26	0.190	0.610	0.122	0.659	0.772	0.780
d 1 to 26	<0.001	0.376	<0.001	0.426	<0.001	0.651
d 27 to 35	<0.001	0.001	<0.001	0.328	0.004	<0.001
d 1 to 35	<0.001	0.002	<0.001	0.080	0.001	0.007
Mortality, %						
d 1 to 17	0.911	0.948	0.823	0.899	0.705	0.827
d 1 to 21	0.977	0.374	0.712	0.569	0.743	0.490
d 18 to 26	0.828	0.428	0.682	0.068	0.474	0.466
d 1 to 26	0.944	0.462	0.898	0.219	0.820	0.845
d 27 to 35	0.595	0.016	0.247	0.008	0.682	0.431
d 1 to 35	0.891	0.163	0.535	0.306	0.669	0.338

[1] Statistical significance was considered at $P \leq 0.05$; Orthogonal polynomial contrasts were performed for the equally spaced Ca concentrations for the main effect and within each limestone PS group.

[2] Limestone was ground using a 2-pair roller mill (Roskamp Champion Series 900-12, California Pellet Mill Co., Crawfordsville, IN) to achieve a PS of 910 µm (coarse) and 200 µm (fine).

Table S5.8. Tibia mineralization responses to calcium (Ca) concentrations as a main effect and within each limestone particle size (PS) group as assessed by orthogonal polynomial contrasts (*P*-values)[1].

	Ca concentration		Limestone particle size[2], µm			
			910		200	
Measurements	Linear	Quadratic	Linear	Quadratic	Linear	Quadratic
Tibia weight, g						
d 21	<0.001	0.145	<0.001	0.095	0.001	0.727
d 35	<0.001	0.019	<0.001	0.400	0.009	0.007
Shear strength, N						
d 21	<0.001	0.927	<0.001	0.407	<0.001	0.484
d 35	<0.001	0.003	<0.001	0.038	<0.001	0.028
Tibia ash, %						
d 21	<0.001	0.923	0.001	0.413	<0.001	0.525
d 35	<0.001	0.077	0.017	0.290	<0.001	0.143

[1] Statistical significance was considered at $P \leq 0.05$; Orthogonal polynomial contrasts were performed for the equally spaced Ca concentrations for the main effect and within each limestone PS group.

[2] Limestone was ground using a 2-pair roller mill (Roskamp Champion Series 900-12, California Pellet Mill Co., Crawfordsville, IN) to achieve a PS of 910 µm (coarse) and 200 µm (fine).

Table S5.9. Nutrient digestibility and apparent ileal digestible energy (AIDE) responses to calcium (Ca) concentrations as a main effect and within each limestone particle size (PS) group as assessed by orthogonal polynomial contrasts (*P*-values)[1].

	Ca concentration		Limestone particle size[2], μm			
			910		200	
Measurements	Linear	Quadratic	Linear	Quadratic	Linear	Quadratic
CP digestibility, %						
d 21	0.608	0.877	0.082	0.396	0.341	0.538
d 35	0.964	0.259	0.391	0.253	0.473	0.640
Fat digestibility, %						
d 21	0.127	0.986	0.012	0.929	0.680	0.937
d 35	0.830	0.458	0.975	0.317	0.785	0.992
Ca digestibility, %						
d 21	<0.001	0.033	<0.001	0.002	<0.001	0.811
d 35	0.001	0.292	0.054	0.109	0.002	0.886
P[3] digestibility, %						
d 21	<0.001	0.449	<0.001	0.350	<0.001	0.041
d 35	<0.001	0.976	0.001	0.472	<0.001	0.511
AIDE[4], kcal/kg						
d 21	0.128	0.057	0.008	0.008	0.525	0.981
d 35	<0.001	0.182	0.001	0.820	0.034	0.109

[1] Statistical significance was considered at $P \leq 0.05$; Orthogonal polynomial contrasts were performed for the equally spaced Ca concentrations for the main effect and within each limestone PS group.

[2] Limestone was ground using a 2-pair roller mill (Roskamp Champion Series 900-12, California Pellet Mill Co., Crawfordsville, IN) to achieve a PS of 910 µm (coarse) and 200 µm (fine).

[3] P = Phosphorus.

[4] On a DM basis.

Table S5.10. Apparent essential and total ileal amino acid (AA) digestibility responses to calcium (Ca) concentrations as a main effect and within each limestone particle size (PS) group as assessed by orthogonal polynomial contrasts (*P*-values)[1].

	Ca concentration		Limestone PS, 910 µm		Limestone PS, 200 µm	
Digestibility, %	Linear	Quadratic	Linear	Quadratic	Linear	Quadratic
Met						
d 21	0.077	0.588	0.004	0.246	0.662	0.057
d 35	0.307	0.541	0.477	0.960	0.460	0.423
Met + Cys						
d 21	0.976	0.861	0.009	0.065	0.010	0.107
d 35	0.141	0.109	0.192	0.391	0.417	0.158
Lys						
d 21	0.265	0.589	0.410	0.483	0.449	0.146
d 35	0.466	0.511	0.790	0.626	0.222	0.658
Thr						
d 21	0.972	0.230	0.323	0.926	0.347	0.080
d 35	0.492	0.688	0.285	0.215	0.070	0.529
Val						
d 21	0.337	0.772	0.299	0.449	0.751	0.245
d 35	0.702	0.251	0.386	0.527	0.195	0.323
Ile						
d 21	0.499	0.770	0.305	0.465	0.941	0.254
d 35	0.735	0.169	0.377	0.336	0.210	0.322
Arg						
d 21	0.380	0.933	0.456	0.603	0.619	0.523
d 35	0.450	0.209	0.628	0.337	0.148	0.409
Trp						
d 21	0.396	0.795	0.815	0.842	0.338	0.573
d 35	0.550	0.794	0.727	0.914	0.263	0.640
Leu						
d 21	0.173	0.850	0.103	0.458	0.770	0.314
d 35	0.972	0.221	0.408	0.303	0.481	0.477
Phe						
d 21	0.233	0.631	0.234	0.532	0.616	0.195
d 35	0.919	0.165	0.347	0.225	0.322	0.444
His						
d 21	0.727	0.852	0.598	0.335	0.973	0.482
d 35	0.702	0.103	0.154	0.201	0.432	0.295
Total AA						
d 21	0.327	0.844	0.246	0.431	0.822	0.288
d 35	0.978	0.163	0.302	0.225	0.367	0.438

[1] Statistical significance was considered at $P \leq 0.05$; Orthogonal polynomial contrasts were performed for the equally spaced Ca concentrations for the main effect and within each limestone PS group.

Table S5.11. Apparent non-essential amino acid digestibility responses to calcium (Ca) concentrations as a main effect and within each limestone particle size (PS) group as assessed by orthogonal polynomial contrasts (*P*-values)[1].

	Ca concentration		Limestone particle size[2], µm			
			910		200	
Digestibility, %	Linear	Quadratic	Linear	Quadratic	Linear	Quadratic
Ala						
d 21	0.094	0.891	0.070	0.407	0.573	0.307
d 35	0.970	0.358	0.418	0.504	0.494	0.526
Asp						
d 21	0.592	0.921	0.566	0.428	0.854	0.351
d 35	0.961	0.070	0.183	0.089	0.202	0.375
Cys						
d 21	0.272	0.299	0.082	0.018	0.003	0.375
d 35	0.001	0.025	0.002	0.062	0.054	0.180
Glu						
d 21	0.312	0.420	0.178	0.920	0.939	0.208
d 35	0.864	0.128	0.250	0.173	0.414	0.418
Gly						
d 21	0.575	0.807	0.380	0.188	0.931	0.329
d 35	0.886	0.177	0.238	0.280	0.378	0.400
Pro						
d 21	0.668	0.768	0.260	0.282	0.599	0.508
d 35	0.441	0.042	0.454	0.041	0.725	0.390
Ser						
d 21	0.257	0.615	0.096	0.127	0.994	0.406
d 35	0.077	0.134	0.048	0.073	0.527	0.721
Tyr						
d 21	0.030	0.688	0.130	0.729	0.113	0.825
d 35	0.390	0.358	0.303	0.185	0.846	0.979

[1] Statistical significance was considered at $P \leq 0.05$; Orthogonal polynomial contrasts were performed for the equally spaced Ca concentrations for the main effect and within each limestone PS group.

[2] Limestone was ground using a 2-pair roller mill (Roskamp Champion Series 900-12, California Pellet Mill Co., Crawfordsville, IN) to achieve a PS of 910 µm (coarse) and 200 µm (fine).

Table S5.12. Digestible nutrient intake (units/bird; DM basis) responses to calcium (Ca) concentrations as a main effect and within each limestone particle size (PS) group as assessed by orthogonal polynomial contrasts (*P*-values)[1].

	Ca concentration		Limestone particle size[2], µm			
			910		200	
Digestible nutrient intake	Linear	Quadratic	Linear	Quadratic	Linear	Quadratic
CP, g						
d 17 to 21	<0.001	0.129	0.086	0.537	<0.001	0.129
d 27 to 35	0.068	<0.001	0.024	<0.001	0.739	0.036
Fat, g						
d 17 to 21	0.916	0.083	0.618	0.088	0.727	0.473
d 27 to 35	0.008	0.142	0.001	0.175	0.658	0.001
Ca, g						
d 17 to 21	0.421	0.018	0.003	<0.001	<0.001	0.082
d 27 to 35	<0.001	0.012	<0.001	<0.001	<0.001	0.769
P[3], g						
d 17 to 21	0.502	0.602	0.072	0.388	0.346	0.905
d 27 to 35	0.001	0.695	<0.001	0.721	0.373	0.363
Calorie, kcal						
d 17 to 21	0.369	0.004	0.514	0.008	0.058	0.141
d 27 to 35	<0.001	<0.001	0.001	0.021	0.001	0.001

[1] Statistical significance was considered at $P \leq 0.05$; Orthogonal polynomial contrasts were performed for the equally spaced Ca concentrations for the main effect and within each limestone PS group.

[2] Limestone was ground using a 2-pair roller mill (Roskamp Champion Series 900-12, California Pellet Mill Co., Crawfordsville, IN) to achieve a PS of 910 µm (coarse) and 200 µm (fine).

[3] P = Phosphorus.

Table S5.13. Digestible essential and total amino acid (AA) intake (units/bird; DM basis) responses to calcium (Ca) concentrations as a main effect and within each limestone particle size (PS) group as assessed by orthogonal polynomial contrasts (*P*-values)[1].

	Ca concentration		Limestone PS, 910 µm		Limestone PS, 200 µm	
Digestible nutrient intake	Linear	Quadratic	Linear	Quadratic	Linear	Quadratic
Met, g						
d 17 to 21	<0.001	0.800	0.036	0.012	<0.001	0.006
d 27 to 35	0.372	<0.001	0.001	0.150	0.063	<0.001
Met + Cys, g						
d 17 to 21	0.001	0.584	0.045	0.024	<0.001	0.004
d 27 to 35	0.020	<0.001	0.248	0.009	0.030	<0.001
Lys, g						
d 17 to 21	<0.001	0.770	<0.001	0.493	0.026	0.795
d 27 to 35	0.881	<0.001	0.118	<0.001	0.093	0.026
Thr, g						
d 17 to 21	<0.001	0.011	<0.001	0.102	0.658	0.043
d 27 to 35	0.041	0.013	0.011	<0.001	<0.001	0.165
Val, g						
d 17 to 21	<0.001	0.800	<0.001	0.589	0.056	0.370
d 27 to 35	0.949	<0.001	0.006	<0.001	0.012	<0.001
Ile, g						
d 17 to 21	<0.001	0.804	<0.001	0.668	0.003	0.433
d 27 to 35	0.864	<0.001	0.006	<0.001	0.016	<0.001
Arg, g						
d 17 to 21	<0.001	0.119	<0.001	0.308	0.009	0.231
d 27 to 35	0.484	<0.001	0.007	<0.001	0.097	0.001
Trp, g						
d 17 to 21	<0.001	0.707	<0.001	0.002	<0.001	<0.001
d 27 to 35	0.141	0.036	0.093	0.430	0.687	0.029
Leu, g						
d 17 to 21	0.010	0.819	0.034	0.536	0.116	0.766
d 27 to 35	0.120	<0.001	0.007	<0.001	0.621	<0.001
Phe, g						
d 17 to 21	<0.001	0.768	<0.001	0.631	0.069	0.370
d 27 to 35	0.177	<0.001	0.008	<0.001	0.444	0.002
His, g						
d 17 to 21	<0.001	0.592	<0.001	0.281	0.020	0.740
d 27 to 35	0.284	<0.001	0.002	<0.001	0.148	<0.001
Total AA, g						
d 17 to 21	0.001	0.891	0.003	0.646	0.047	0.517
d 27 to 35	0.290	<0.001	0.007	<0.001	0.230	0.001

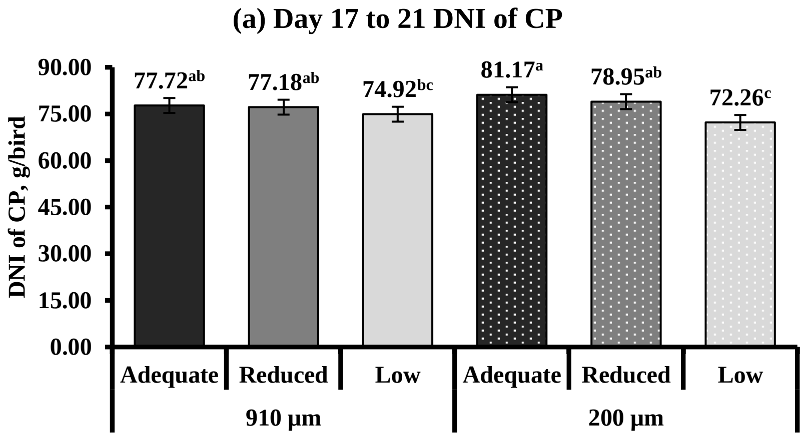
[1] Statistical significance was considered at $P \leq 0.05$; Orthogonal polynomial contrasts were performed for the equally spaced Ca concentrations for the main effect and within each limestone PS group.

Table S5.14. Digestible non-essential amino acid intake (units/bird; DM basis) responses to calcium (Ca) concentrations as a main effect and within each limestone particle size (PS) group as assessed by orthogonal polynomial contrasts (*P*-values)[1].

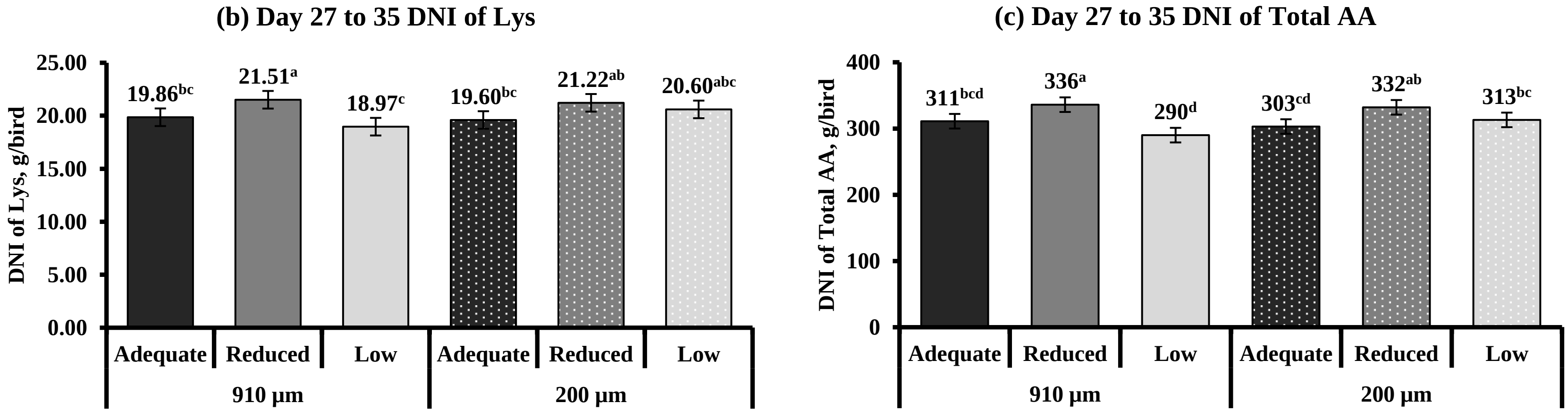
	Ca concentration		Limestone particle size[2], µm			
			910		200	
Digestible nutrient intake	Linear	Quadratic	Linear	Quadratic	Linear	Quadratic
Ala, g						
d 17 to 21	0.045	0.719	0.190	0.518	0.121	0.891
d 27 to 35	0.064	<0.001	0.013	0.001	0.894	0.001
Asp, g						
d 17 to 21	<0.001	0.690	<0.001	0.597	0.087	0.284
d 27 to 35	0.210	<0.001	0.001	<0.001	0.101	0.002
Cys, g						
d 17 to 21	0.002	0.291	0.214	0.011	<0.001	0.269
d 27 to 35	<0.001	<0.001	<0.001	<0.001	0.034	0.007
Glu, g						
d 17 to 21	<0.001	0.534	<0.001	0.647	0.013	0.671
d 27 to 35	0.011	<0.001	<0.001	<0.001	0.928	<0.001
Gly, g						
d 17 to 21	<0.001	0.557	0.003	0.166	0.014	0.565
d 27 to 35	0.758	<0.001	0.041	<0.001	0.124	0.003
Pro, g						
d 17 to 21	0.001	0.662	0.009	0.384	0.024	0.802
d 27 to 35	<0.001	<0.001	<0.001	<0.001	0.203	<0.001
Ser, g						
d 17 to 21	0.134	0.933	0.197	0.410	0.393	0.356
d 27 to 35	<0.001	<0.001	<0.001	<0.001	0.002	0.093
Tyr, g						
d 17 to 21	0.041	0.080	0.001	0.794	0.622	0.028
d 27 to 35	0.002	<0.001	0.001	<0.001	0.304	0.003

[1] Statistical significance was considered at $P \leq 0.05$; Orthogonal polynomial contrasts were performed for the equally spaced Ca concentrations for the main effect and within each limestone PS group.

[2] Limestone was ground using a 2-pair roller mill (Roskamp Champion Series 900-12, California Pellet Mill Co., Crawfordsville, IN) to achieve a PS of 910 µm (coarse) and 200 µm (fine).



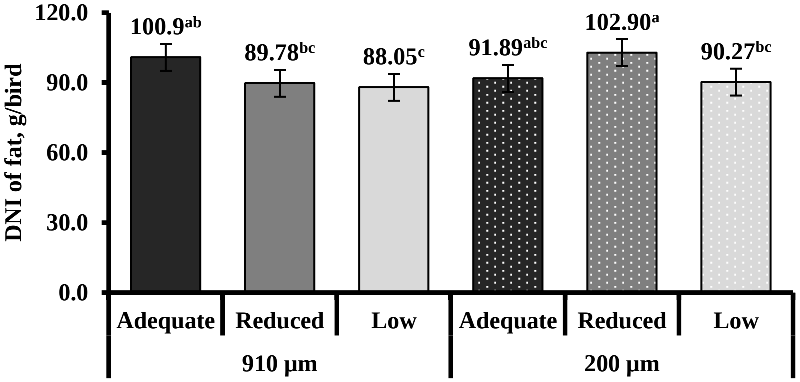
(a) Day 17 to 21 DNI of CP

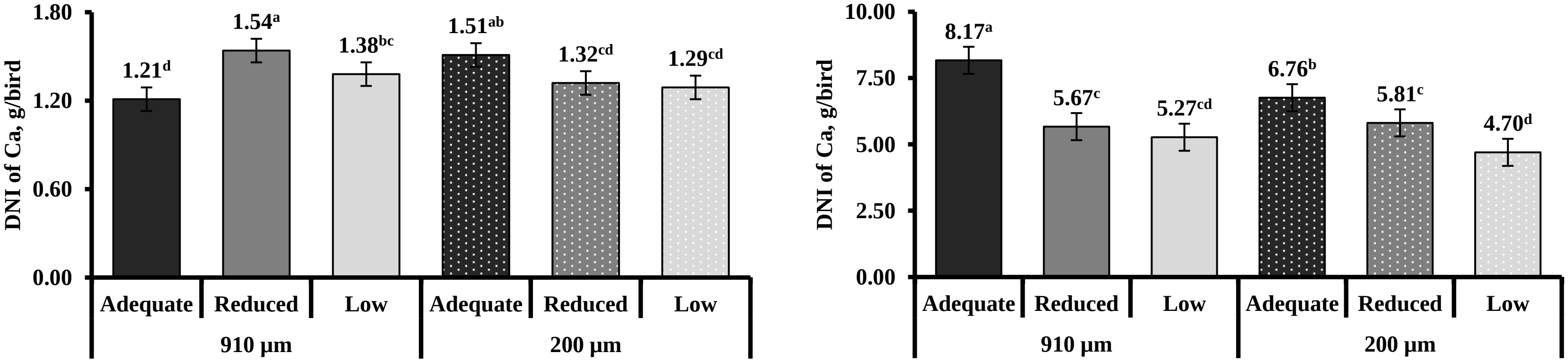


(b) Day 27 to 35 DNI of Lys

(c) Day 27 to 35 DNI of Total AA

Figure S5.1. Interaction effects[1] of varying limestone particle size (910 and 200 µm) and Ca concentrations (adequate, reduced, and low) fed to YPM x Ross 708 male broilers on (**a**) d 17 to 21 DNI of CP (*P* = 0.031; CLM ± 2.40), (**b**) d 27 to 35 DNI of Lys (*P* = 0.030; CLM ± 0.83), and (**c**) d 27 to 35 DNI of total AA (*P* = 0.012; CLM ± 11). [a-d]Means with different superscripts differ significantly ($P \leq 0.05$). [1]Interaction values are least square means of 10 replicate pens.


(a) Day 27 to 35 DNI of Fat


(b) Day 17 to 21 DNI of Ca

(c) Day 27 to 35 DNI of Ca

Figure S5.2. Interaction effects[1] of varying limestone particle size (910 and 200 µm) and Ca concentrations (adequate, reduced, and low) fed to YPM x Ross 708 male broilers on (**a**) d 27 to 35 DNI of fat ($P = 0.001$; CLM ± 5.77), (**b**) d 17 to 21 DNI of Ca ($P < 0.001$; CLM ± 0.08), and (**c**) d 27 to 35 DNI of Ca ($P = 0.005$; CLM ± 0.51). $^{a\text{-}d}$Means with different superscripts differ significantly ($P \leq 0.05$). [1]Interaction values are least square means of 10 replicate pens.

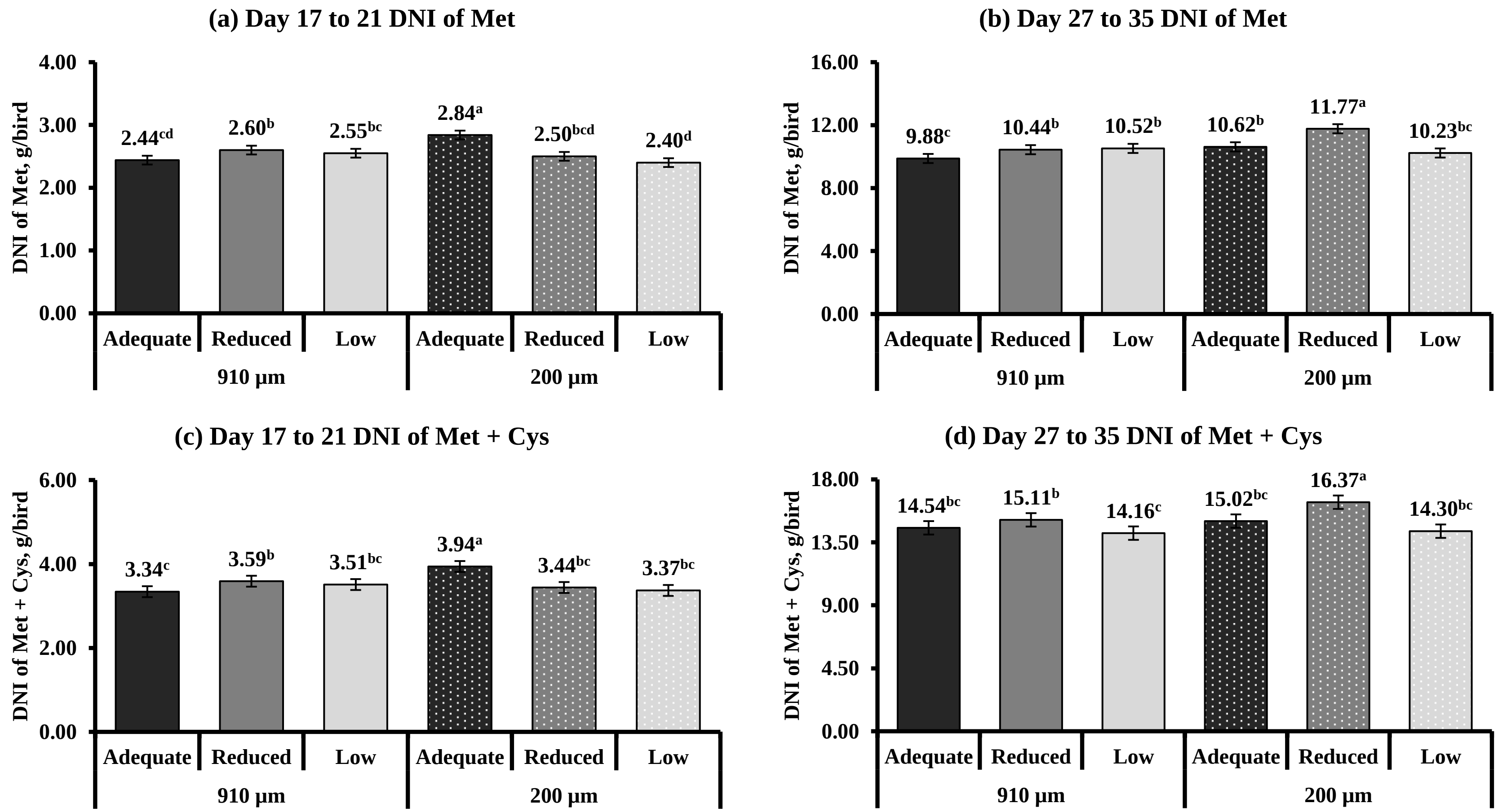
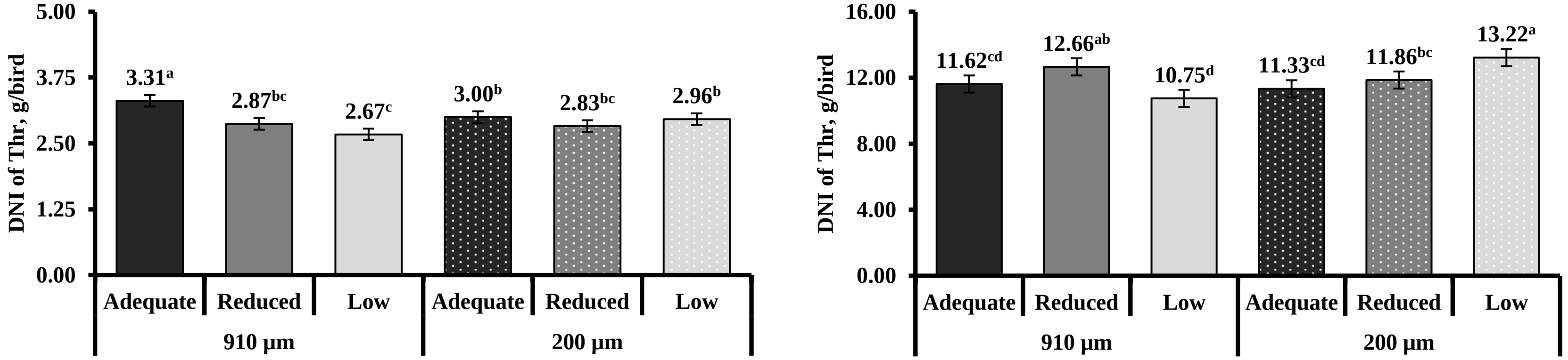


Figure S5.3. Interaction effects[1] of varying limestone particle size (910 and 200 µm) and Ca concentrations (adequate, reduced, and low) fed to male broilers on (**a**) d 17 to 21 DNI of Met ($P < 0.001$; CLM ± 0.07), (**b**) d 27 to 35 DNI of Met ($P < 0.001$; CLM ± 0.29), (**c**) d 17 to 21 DNI of Met + Cys ($P < 0.001$; CLM ± 0.13), and (**d**) d 27 to 35 DNI of Met + Cys ($P = 0.040$; CLM ± 0.48). $^{a\text{-}d}$Means with different superscripts differ significantly ($P \leq 0.05$). [1]Interaction values are least square means of 10 replicate pens.

(a) Day 17 to 21 DNI of Thr

(b) Day 27 to 35 DNI of Thr



(c) Day 27 to 35 DNI of Val

(d) Day 27 to 35 DNI of Ile

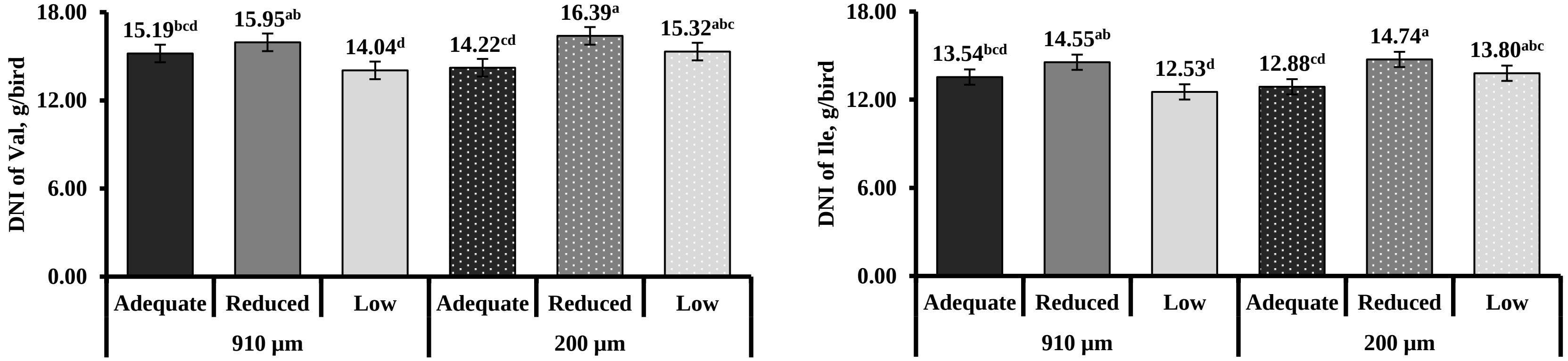
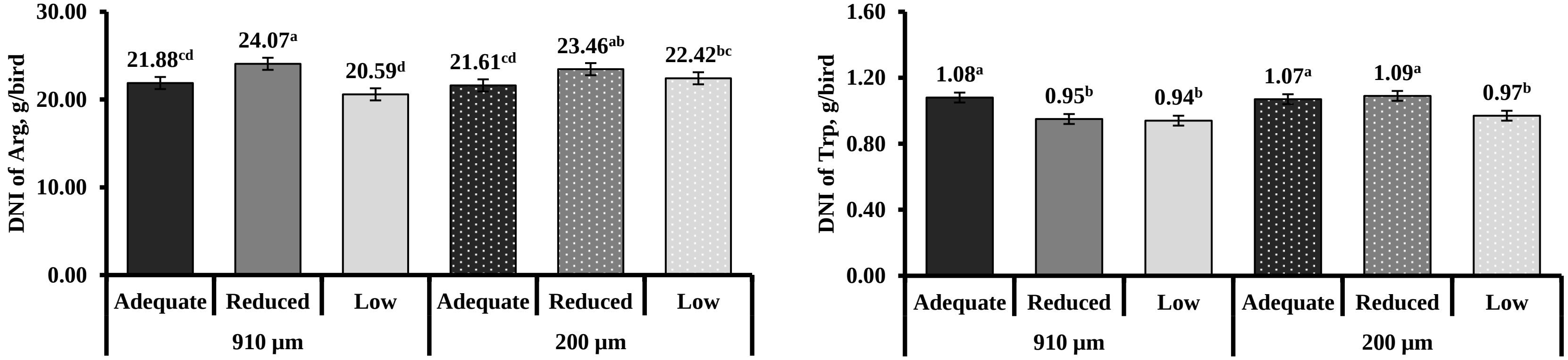
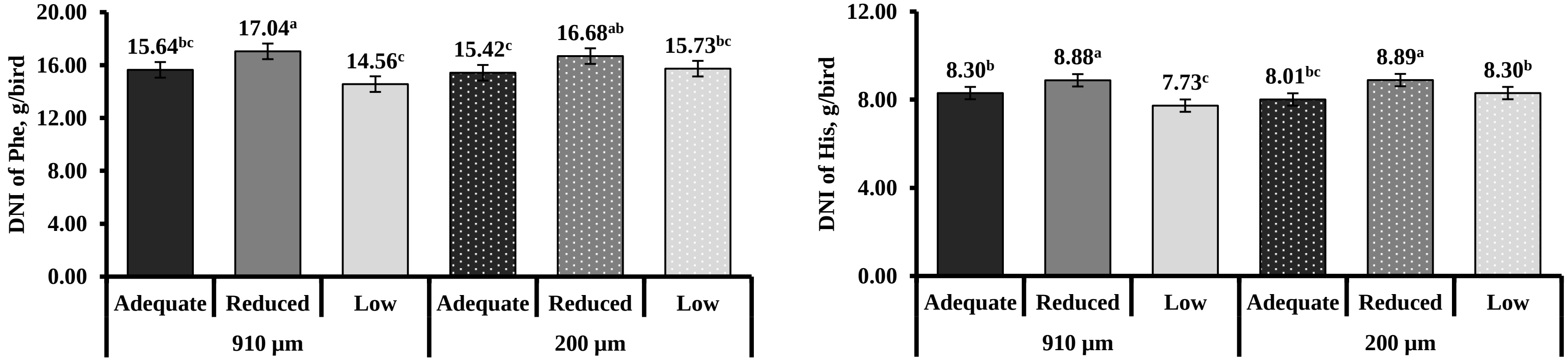


Figure S5.4. Interaction effects[1] of varying limestone particle size (910 and 200 µm) and Ca concentrations (adequate, reduced, and low) fed to YPM x Ross 708 male broilers on (**a**) d 17 to 21 DNI of Thr ($P < 0.001$; CLM ± 0.11), (**b**) d 27 to 35 DNI of Thr ($P < 0.001$; CLM ± 0.52), (**c**) d 27 to 35 DNI of Val ($P = 0.001$; CLM ± 0.60), and (**d**) d 27 to 35 DNI of Ile ($P = 0.002$; CLM ± 0.52). $^{a\text{-}d}$Means with different superscripts differ significantly ($P \leq 0.05$). [1]Interaction values are least square means of 10 replicate pens.



(a) Day 27 to 35 DNI of Arg

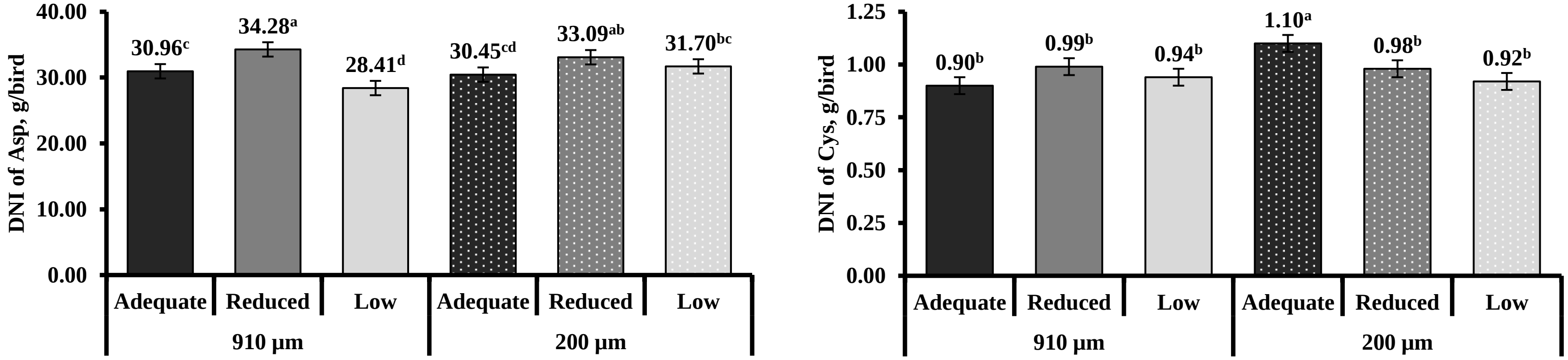
(b) Day 17 to 21 DNI of Trp



(c) Day 27 to 35 DNI of Phe

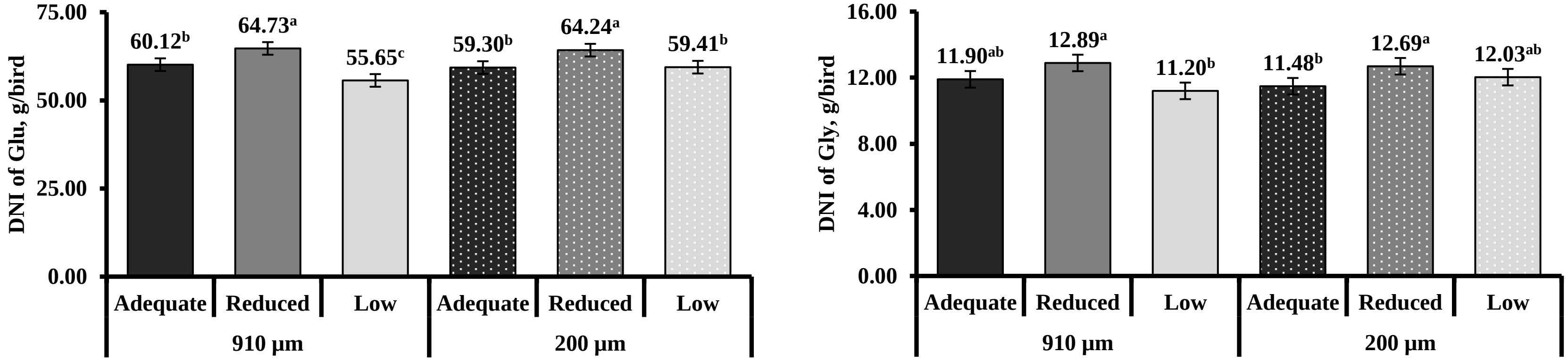
(d) Day 27 to 35 DNI of His

Figure S5.5. Interaction effects[1] of varying limestone particle size (910 and 200 µm) and Ca concentrations (adequate, reduced, and low) fed to YPM x Ross 708 male broilers on (**a**) d 27 to 35 DNI of Arg ($P = 0.001$; CLM ± 0.69), (**b**) d 17 to 21 DNI of Trp ($P < 0.001$; CLM ± 0.03), (**c**) d 27 to 35 DNI of Phe ($P = 0.016$; CLM ± 0.59), and (**d**) d 27 to 35 DNI of His ($P = 0.007$; CLM ± 0.28). [a-d]Means with different superscripts differ significantly ($P \leq 0.05$). [1]Interaction values are least square means of 10 replicate pens.



(a) Day 27 to 35 DNI of Asp

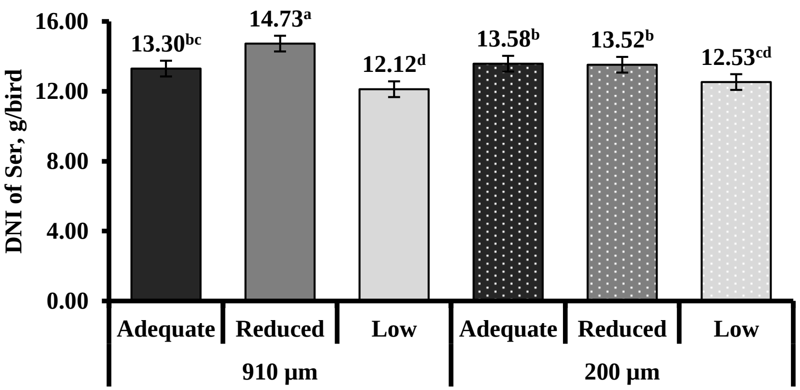
(b) Day 17 to 21 DNI of Cys

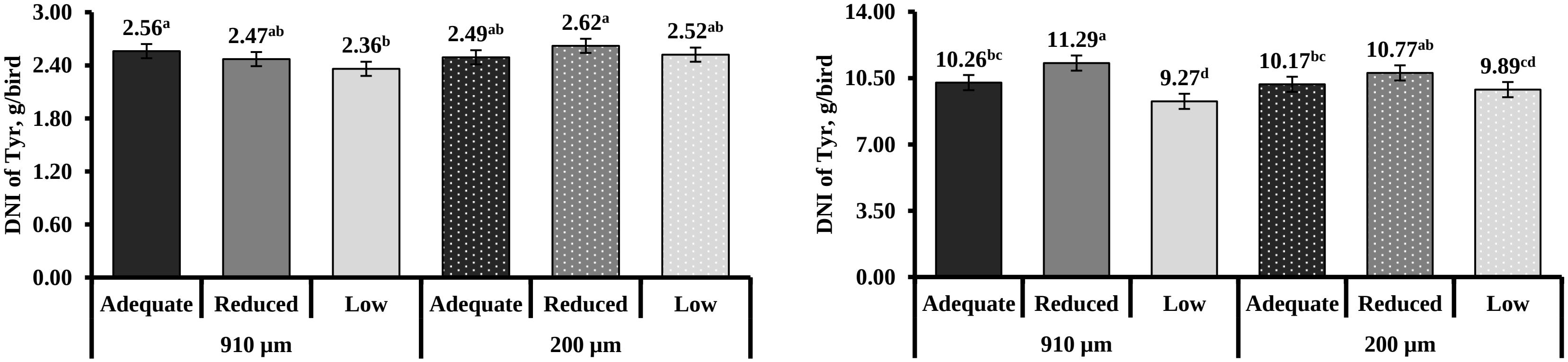


(c) Day 27 to 35 DNI of Glu

(d) Day 27 to 35 DNI of Gly

Figure S5.6. Interaction effects[1] of varying limestone particle size (910 and 200 µm) and Ca concentrations (adequate, reduced, and low) fed to YPM x Ross 708 male broilers on (**a**) d 27 to 35 DNI of Asp (*P* < 0.001; CLM ± 1.09), (**b**) d 17 to 21 DNI of Cys (*P* < 0.001; CLM ± 0.04), (**c**) d 27 to 35 DNI of Glu (*P* = 0.011; CLM ± 1.79), and (**d**) d 27 to 35 DNI of Gly (*P* = 0.028; CLM ± 0.50). $^{a\text{-}d}$Means with different superscripts differ significantly (*P* ≤ 0.05). [1]Interaction values are least square means of 10 replicate pens.


(a) Day 27 to 35 DNI of Ser


(b) Day 17 to 21 DNI of Tyr

(c) Day 27 to 35 DNI of Tyr

Figure S5.7. Interaction effects[1] of varying limestone particle size (910 and 200 µm) and Ca concentrations (adequate, reduced, and low) fed to YPM x Ross 708 male broilers on (**a**) d 27 to 35 DNI of Ser (*P* = 0.001; CLM ± 0.45), (**b**) d 17 to 21 DNI of Tyr (*P* = 0.010; CLM ± 0.08), and (**c**) d 27 to 35 DNI of Tyr (*P* = 0.019; CLM ± 0.40). $^{a\text{-}d}$Means with different superscripts differ significantly ($P \leq 0.05$). [1] Interaction values are least square means of 10 replicate pens.