Evaluation of fungal lysozymes in the growth performance of broiler chickens

Ebenezer Amponsem Boateng

Department of Bioresource Engineering

McGill UNIVERSITY

Faculty of Agricultural and Environmental Sciences

McGill University, Montreal

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DEDICATION

This thesis is dedicated to my family and friends for their love, care, advice, and support throughout my studies.

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"And I am sure of this, that he who began a good work in you will bring it to completion at the day of Jesus Christ" (Philippians 1:6).

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May God richly bless you all.

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PREFACE

This thesis was written following the "Guidelines for Thesis Preparation" requirements by McGill University. This thesis has an abstract in English and French and contains six chapters: an introduction, a comprehensive review of the literature, materials and methods, results, discussion, and conclusion.

Contribution of Co-Authors

All the six chapters in the thesis were authored by Ebenezer Amponsem Boateng (principal author) under the supervision of Dr. Michael O. Ngadi and Dr. Jennifer Ronholm. Dr. Ngadi provided scientific and technical advice for the research and review sections of the thesis. Dr. Ronholm guided the various sections of the thesis and ensured the thesis met standards by providing several suggestions and technical questions as well as reviewing all the sections of this thesis. Dr. Roger I. Cue reviewed chapters one and three of this thesis and provided technical guidance in analyzing the data using the SAS software and statistical modeling.

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LIST OF ABBREVIATIONS

ABR	Antibiotic resistance
ACC	Animal Care Committee
ADG	Average daily gain
AGPs	Antibiotic growth promoters
AI	Avian influenza
AMR	Antimicrobial resistance
ANOVA	Analysis of variance
APEC	Avian Pathogenic *Escherichia coli*
BMD	Bacitracin methylene disalicylate
BWG	Bodyweight gain
CIPARS	Canadian Integrated Program for Antimicrobial Resistance Surveillance
CP	Crude protein
DFM	Direct-fed microbial
DOCs	Day-old chicks
EBI	European broiler index
ECDC	European Center for Disease Prevention and Control
EU	European union
EPEF	European production efficiency factor

ESVAC	European Surveillance of Veterinary Antimicrobial Consumption
ExPEC	Extraintestinal pathogenic *Escherichia coli*
FAES	Faculty of Agricultural and Environmental Sciences
FCR	Feed conversion ratio
FOS	Fructooligosaccharides
FW	Final bodyweight
GIT	Gastrointestinal tract
GLM	Generalized linear model
IW	Initial bodyweight
MDR	Multi-drug resistant
MOS	Mannan-oligosaccharides
NRC	National Research Council
NSP	Non-starch polysaccharides
PFAs	Phytogenic feed additives
PHAC	Public Health Agency of Canada
PRRS	Porcine reproductive and respiratory syndrome
RCBD	Randomized complete block design
SAA	Sulfur-containing amino acids

SAS	Statistical analysis software
SEM	Standard error of the mean
SFP	Single floor pen
TFI	Total feed intake
TWG	Total weight gain
USDA	United States Department of Agriculture
VRE	Vancomycin-resistant enterococci
WHO	World health organization

ABSTRACT

The intensification of poultry production to meet the supply and demand of poultry meat has led to the use of antibiotics as growth promoters for many years leading to problems associated with antibiotic resistance and residues in meat and meat products which consequently affect the ecosystem. A six-week trial was conducted to determine the growth response of broilers fed different lysozyme candidates as a replacement for conventional antibiotics (Bacitracin methylene disalicylate (BMD)) as growth promoters. The treatment period began after a 2-week starter phase. One hundred and sixty (160) male Ross 308 broilers were randomly allotted to eight treatments by bodyweight in a randomized complete block design (RCBD). The treatments included a basal diet (T1 = control diet which was free from antibiotics or other growth promoters), and the basal diet supplemented with six different fungal lysozymes at a concentration of 30 mg/kg feed: LYZ25A_RHISO (T2), LYZ25A_NEOFI (T3), LYZ25A_TALPR (T4), LYZ25A_PENCH (T5), LYZ25A_MYCHI (T6) and LYZ25A_POCCH (T7) as well as the basal diet supplemented with a conventional antibiotic, BMD (T8) at an inclusion rate of 55 mg/kg feed. Each treatment had ten replicates consisting of two birds per replicate. Broiler starter and grower diets (mash) were formulated by Belisle Solutions Inc. (St-Mathias-sur-Richelieu, QC). Feed and water were provided *ad-libitum.* All birds were euthanized on the last day of the trial for further investigations. Among the growth parameters measured were initial bodyweight, feed intake, weight gain, and feed conversion ratio. The data obtained were analyzed using the PROC GLM procedure of SAS (SAS Institute Inc., Cary, NC, USA). Differences in experimental treatments were tested using Tukey Pairwise Comparisons and after analysis of variance (ANOVA). All results from the data were considered to be different significantly at $P < 0.05$. The birds supplemented with the test fungal lysozymes (T2 to T7) had no significant differences ($P > 0.05$) on any of the performance

parameters observed when compared to the control (T1) and the antibiotic-containing diet (T8). Numerically, the current study results indicated that the dietary addition of lysozymes, especially NEOFI and POCCH could improve broilers' performance, which will contribute to food security and safety. Therefore, it was concluded that fungal lysozymes (especially LYZ25A_NEOFI and LYZ25A_POCCH) could be used to replace antibiotics (BMD) in the diets of broilers.

RÉSUMÉ

L'intensification de la production de volaille pour répondre à l'offre et à la demande de viande de volaille a conduit à l'utilisation d'antibiotiques comme facteurs de croissance depuis de nombreuses années, ce qui a entraîné des problèmes liés à la résistance aux antibiotiques et aux résidus dans la viande et les produits carnés qui affectent par conséquent l'écosystème. Un essai de six semaines a été mené pour déterminer la réaction de croissance des poulets de chair nourris de différents lysozymes substituant les antibiotiques classiques (Bacitracine méthylène disalicylate (BMD)) comme promoteurs de croissance. La période de traitement a commencé après une phase de démarrage de 2 semaines. Cent soixante (160) poulets de chair mâles Ross 308 ont été répartis au hasard dans huit traitements en fonction du poids corporel selon un modèle de bloc complet randomisé (RCBD). Les traitements comprenaient le régime de base (T1 = régime témoin exempt d'antibiotiques ou d'autres facteurs de croissance), et le régime de base complété par six lysozymes fongiques différents à une concentration de 30 mg/kg d'aliment : LYZ25A_RHISO (T2), LYZ25A_NEOFI (T3), LYZ25A_TALPR (T4), LYZ25A_PENCH (T5), LYZ25A_MYCHI (T6) et LYZ25A_POCCH (T7) ainsi que le régime de base complété par un antibiotique conventionnel, la BMD (T8) à un taux d'inclusion de 55 mg/kg d'aliment. Chaque traitement avait dix répétitions composées de deux oiseaux par répétition. Les régimes de démarrage et de croissance (purée) pour poulets de chair ont été formulés par Belisle Solutions Inc. (St-Mathias-sur-Richelieu, QC. Des aliments et de l'eau ont été fournis ad-libitum (à volonté). Tous les oiseaux ont été euthanasiés le dernier jour de l'essai pour des enquêtes plus approfondies. Parmi les paramètres de croissance mesurés figuraient le poids corporel initial, l'apport alimentaire, le gain de poids et le rapport de conversion alimentaire. Les données obtenues ont été analysées à l'aide de la procédure PROC GLM de SAS (SAS Institute Inc., Cary, NC, USA). Les différences dans les traitements

expérimentaux ont été testées à l'aide de comparaisons par paires de Tukey et après analyse de la variance (ANOVA). Tous les résultats des données ont été considérés comme significativement différents à $P < 0,05$. Les oiseaux supplémentés avec les lysozymes fongiques testés (T2 à T7) n'avaient aucune différence significative ($P > 0,05$) sur aucun des paramètres de performance observés par rapport au contrôle (T1) et au régime contenant des antibiotiques (T8). Numériquement, les résultats de la présente étude ont indiqué que l'ajout de lysozymes dans l'alimentation, en particulier NEOFI et POCCH, pourrait améliorer le rendement des poulets de chair, ce qui contribuera à la sécurité et à la salubrité alimentaires. Par conséquent, il a été conclu que les lysozymes fongiques (en particulier LYZ25A_NEOFI et LYZ25A_POCCH) pourraient être utilisés pour remplacer les antibiotiques (BMD) dans l'alimentation des poulets de chair.

CHAPTER 1. INTRODUCTION

1.1 General Introduction

Chickens constitute the majority (63%) of all avian breeds used in food production, with ducks accounting for 11%, and geese and turkeys accounting for 9 and 5%, respectively (Pym 2013) The poultry sector has increased in size globally and is possibly the fastest growing and most flexible of all livestock production sectors (Mcleod et al. 2015). Intensification of poultry production to ensure food security has led to the demand for growth promotion products. Antibiotics are used to help prevent disease, combat bacterial infections and promote animal growth (Diarra and Malouin 2014; Van Boekel et al. 2015). However, the excessive use of antibiotics as growth promoters has resulted in the proliferation of antibiotic resistance (ABR) with the potential for the transfer of genes for ABR from animal to human microbiota (Mingmongkolchai and Panbangred 2018). It is estimated that by 2050, 10 million people will die annually due to antimicrobial-resistant infections (Sugden et al. 2016); a fact that highlights the need to reduce antibiotic use in agriculture. There is the potential use of non-antibiotic alternatives such as probiotics, prebiotics, organic acids, in animal production as growth promoters (Castanon 2007; Edens 2003; Patterson and Burkholder 2003; Guo et al. 2004). In this project, we are evaluating if lysozymes can potentially replace antibiotics, specifically BMD, for non-therapeutic growth promotion in broiler chickens.

1.2 Rationale

Antibiotics are used to achieve high productivity in the poultry industry, but this has led to the resistance of several bacterial pathogens to the same antibiotics, and this practice is no longer sustainable. Lysozyme is a potent antimicrobial protein found in nature that plays an important

role in the innate immune system and is, therefore, a potential growth promoter in animal production. This work will examine the use of fungal lysozymes as an alternative to antibiotics in the diets of broiler chickens.

1.3 Research Hypothesis

Lysozymes degrade the peptidoglycan of Gram-positive bacteria that are responsible for necrotic enteritis in chickens; therefore, certain lysozymes will have growth promotion abilities comparable to BMD in broilers.

1.4 Research Objective

1 – Conduct a broiler growth trial of six potential growth-promoting lysozymes over a 42-day growth period.

2 – Statistically compare the growth-promoting effects of each of the six lysozymes to bacitracin methylene disalicylate (BMD) (positive control), and un-supplemented food (negative control).

CHAPTER 2. LITERATURE REVIEW

2.1 Introduction to poultry and poultry production

Gallus gallus domesticus, commonly known as the chicken and routinely used for food production, was domesticated from wild jungle fowl species including *Gallus gallus* and *Gallus varius* around 2000 B.C. (Arrebola et al. 2018; Boggia et al. 2019). Before industrialization, the main concern of breeders was with various body features such as colouring. However, modern domestication goals have primarily been driven by human demands for efficient productivity, liveability, and wellbeing (Bródka et al. 2012). The modern domestic chicken is currently raised industrially in almost every climate on Earth including arctic, sub-arctic, temperate, and tropical zones (Arrebola et al. 2018).

Poultry is the most produced meat in terms of volume, and production volume is currently the highest in the USA, China, and Brazil (Conway 2018). The introduction of cut parts and frozen ingredients is another factor contributing to the rise in poultry consumption. Poultry consumption has largely evolved around two forms of market benefits: reduced cooking time (e.g., ready-to-eat) and increased variety of consumption locations (e.g., fast-food restaurants). Outside-the-home catering incorporates all of these elements. In comparison to nearly 50% in the late 1980s, whole chickens today account for a relatively small portion of the total market. For example, in the USA, poultry sold as whole carcasses, cut-up parts, or processed products represented 9, 39, and 51%, respectively, in 2019 (USDA 2019). Looking at these proportions in France, it was 22, 48, and 29%, respectively, in 2018 (Francaise 2018).

Chicken production has undergone a great improvement within the past 5 decades, with increases in productivity due to better technology and management strategies (Costantino et al. 2018). Specifically, genetic selection, nutritional improvements, and proper biocontrol have led to

increases in the production and supply of poultry to consumers over the past decade (Kryeziu et al. 2018; Namakparvar et al. 2014) The need for improvements in growth and utilization of feed is addressed in broiler breeding (Jahanpour et al. 2014). Factors that affect economic success on-farm include the feeding system, strain selection, flock reactions to diseases and metabolic disorders, stocking density, the best slaughter age, and proper breeding capacity (Berg and Yngvesson 2012; Kryeziu et al. 2018). Environmental stressors as a result of poor stocking density, humidity, and temperature are part of the main factors influencing the performance of the chicken industry (Pompeu et al. 2018).

2.2 Challenges in the Chicken Industry

2.2.1 Climate Change

Poultry health and immunity, and climate change are the biggest challenges to the growth of the industry. Broiler growth characteristics may be affected by the evolution and the climate in particular geographical locations (Okere 2014). For example, poultry production in tropical locations such as Africa faces a major challenge due to heat stress which is associated with climate change, and it negatively affects the growth and production performance of poultry. As a result, small-scale poultry farmers usually invest in alternative traditional approaches such as the use of local breeds while medium to large scale farmers use modern technology like energy-saving light bulbs that produce less heat as well as water and air ventilation systems (Liverpool-Tasie et al. 2019). Climate change and animal production are linked, and the effects of climate change on livestock and poultry production are evident all over the world (Mengesha 2011). For example, when the temperature is above 32°C, the feed intake of broiler chickens is reduced by 5% for each degree of temperature increase (Balogun et al. 2013).

Climate change is a major challenge for small-scale poultry farmers using open-house systems especially in the tropics (Oloyo and Ojerinde 2019). This system has structures that allow natural ventilation and lighting and hence, no need for the regulation of temperature and humidity but partial control of lighting especially for laying hens. Climate change leads to an increasing temperature which requires most farmers in the tropics to design their poultry structures to suit natural ventilation (open-house) but as a result, this system has further affected the maintenance of biosecurity due to the problems related to getting rid of wild birds and disease-carrying rodents off the poultry farm (Cole and Desphande 2019). Different breeds and ages of poultry respond to climatic changes in different ways (Alade and Ademola 2013). For example, Hubbard, Arbor Acres and Ross commercial breeds native to mild climates of Europe and North America and are generally not resistant to warm to hot climatic conditions. Indigenous breeds and locally bred variations of the foreign breeds tend to tolerate higher temperatures when compared to these commercial breeds (Cole and Desphande 2019).

2.2.2 Bans on the Use of Antibiotics

In a recent study, environmental challenges were found to be the main problem faced by poultry farmers in Brazil, and low feed conversion was the second most concerning issue (Mendes et al. 2014). Farmers are concerned about the feed conversion ratio because feed costs may account for up to 70% of the overall cost of poultry production. As a result, poultry producers go to great lengths to reach optimum productivity in balancing diets in order to increase feed conversion ratios (Mendes et al. 2014). The use of antibiotic growth promoters (AGPs) in broilers can increase bodyweight by up to 8% and improve feed conversion by about 5% (Feighner and Dashkevicz 1987). However, in response to the growing ABR crisis several governments around the world

have taken steps to ban the use of antibiotics for growth promotion starting with Sweden in 1986 (Chabot et al. 2015), and most recently Vietnam in 2020 (Ward 2016). While these bans may benefit producers with good on-farm biocontrol, the same bans may be devastating to the productivity and profitability of some farms that currently rely on antibiotics to compensate for poor hygiene (Council 1999).

Multi-drug resistant (MDR) bacteria are a major threat to the welfare and productivity of farmed animals. For example, the antimicrobial susceptibility of 145 *Enterococcus* strains from poultry was investigated by Maasjost et al. (2015). A total of eighty-nine isolates were found to be tolerant to three or more antimicrobials. Lincomycin, tetracycline, and gentamicin resistance are the most prevalent in *Enterococcus faecalis* isolated from chicken (Maasjost et al. 2015) In a second study analysing antimicrobial resistance (AMR) in *Campylobacter jejuni* isolates from turkeys, just one isolate from 76 was sensitive to all antibiotics tested (El-Adawy et al. 2012). Amoxicillin tolerance was found in forty-four (57.9%), streptomycin tolerance in sixty-nine (90.8%), erythromycin tolerance in sixty-one (80.2%), and neomycin tolerance in fifty-eight (76.4%) of the isolates (El-Adawy et al. 2012). Sulfamethoxazole/trimethoprim, metronidazole, ciprofloxacin, nalidixic acid, and tetracycline resistance was respectively found to be fifty-eight (76.3%), fifty-eight (76.3%), fifty-three (69.7%), fifty-one (67.1%) and forty-two (55.3%) (El-Adawy et al. 2012).

2.2.3 Challenges of Poultry Production in the Developing World

The developing world faces different problems in relation to poultry production than the developed world. For example, in Ethiopia 5 major constraints to poultry production were identified: sudden disease outbreak, high costs of commercial rations, unavailability of day-old chicks at the appropriate time, market instability, and poor supply and quality of vaccines (Ebsa et

al. 2019). Lack of access to credit and insufficient training were also identified as major challenges facing chicken farmers specifically in Addis Ababa (Nebiyu 2016). The lack of knowledge on feed composition and compounding, the high price of mixed feed, the lack of commercial feed in a nearby area, and the unavailability and cost of feed ingredients were all major production limitations in Tigray (also Ethiopia) when operating in an intensive system (Tadesse et al. 2017). In addition, Kuwait's poultry industry relies on the import of main feed ingredients such as soybeans and corn from the United States of America and India as well as sourcing day-old chicks (DOCs) for broiler or layer production from hatcheries from Europe (Al-Nasser et al. 2015). This has resulted in a higher cost of production, reduced production efficiency, animal health monitoring, changes in trade policies and practices amongst others as the main challenges the poultry industry faces in Kuwait (Al-Nasser et al. 2015).

2.3 Broiler Nutrition

Animal nutrition plays a very important role and feed cost accounts for about 70 to 80% of all chicken production costs (Willems et al. 2013; Mottet et al. 2017; Ronquillo and Hernandez 2017). Feed management should be mindful of how to improve feed quality and manage feed costs in animal production (Tullo et al. 2019). Various commercial feed mills now provide different types of broiler feed for different age classes of birds. Broiler meat yield is influenced by the physical form of the feed (mash, pellet, or crumble) (Shabani et al. 2015). The conversion of animal feed into poultry meat is the primary goal of chicken nutrition. Mash is a form of complete diet that has been finely ground and compounded to prevent chickens from quickly separating ingredients which ensures a balanced diet with each mouthful. Pelleting is a modified form of mash. It entails pressing the mash mechanically into a hard and dry product, often known as

"artificial grains". Pellets are made from compacted mash that has been extruded to a diameter of about 1/8 inch and a length of 1/4 inch. Trials have been undertaken to determine if the type of feed form has an effect on broiler growth performance, and significant ($P<0.05$) differences among the three feed forms in the 3rd to 6th week of feed intake were found (Chehraghi et al. 2013). The crumble, pellet, and mash categories had the maximum, average and lowest, feed consumption, respectively (Chehraghi et al. 2013). Other studies have reported similar findings (Allerd et al. 1996).

Broilers have been highly selected for higher feed conversion efficiency and improved yield of breast meat (Zuidhof et al. 2014). Voluntary feed intake is the major driver of growth in broilers; however, *ad libitum* feeding with long-day lengths may have adverse consequences to the birds, namely: overconsumption of feed which can negatively affect feed efficiency, nutrient digestibility, and induction of physiological growth-related problems (Schwean-Lardner et al. 2013). Broiler selection has resulted in significant improvements in their body structure and, as a result, in their nutritional needs, with an emphasis on amino acid requirements (Zampiga et al. 2018). Current commercial feeds for broilers, for example, have higher lysine levels than those previously suggested by the National Research Council (NRC) to promote exceptional breast muscle growth in modern genetic lines (Zampiga et al. 2018).

Dietary nutrient manipulation, specifically protein manipulation, plays a major role in breast meat yield in broilers (Horniakova and Abas 2009). Broiler carcass composition, especially breast meat yield, has been shown to benefit from sulfur-containing amino acids (SAA) and lysine (Vieira et al. 2004). Additionally, these amino acids have the ability for reducing the accumulation of abdominal fat (Nasr and Kheiri 2011). An improved weight gain with FCR was observed when lysine and methionine levels were set at 130 percent of the NRC requirement and issued to the

broiler for 42 days (Ahmed and Abbas 2011). Higher weights for birds fed increasing (0.38, 0.44, and 0.50%) levels of Methionine were also reported (Kalvandi et al. 2019). Improved bodyweight in 28- and 48-day old broilers can be observed when higher levels of lysine are added to their diet (Jia et al. 2019). An increase in weight and a daily diet of birds feeding on elevated Lysine food levels were observed (Ishii et al. 2019).

In broiler production, energy consumption is very important because it has a direct effect on growth rate and carcass characteristics, it is also linked to metabolic disorders such as fatty liver syndrome and ascites (Maharjan et al. 2020). Therefore, the focus is often placed on the levels of inclusion in various energy sources when developing feed for chicken, because an increase or decrease in dietary energy can play an important role in determining not only the cost but also final product quality (Dozier III and Gehring 2014). The nutrient density in the diet should be adjusted to allow for the nutritious intake according to the needs of the actual feed intake. The energy requirement of the different phases of growth of broilers was reported as 3000 kcal ME/kg or 12.55 MJ/kg for starters; 3100 kcal ME/kg or 12.97 MJ/kg for growers and 3200 kcal ME/kg or 13.39 MJ/kg for finishers (Aviagen 2014). Chickens that are fed with a low-energy diet consume significantly more feed than chickens fed with control and high-energy diets (Classen 2013). In addition, chickens that are fed with low-energy diets or diets that had elevated non-starch polysaccharides (NSP) consume more feed, compared with hens that were fed the control diets (Maynard et al. 2019). With distinct broiler chicken strains, research has indicated diverse reactions to energy concentration (Kim et al. 2012).

2.4 Feed Conversion Ratio (FCR)

An efficient way to reduce the cost of feed is to increase the level of feed utilization. FCR is a key metric for determining the efficiency of chicken feed consumption. It is measured by feed

intake and BWG and is a non-standard equivalent measure. With increasing complex fluctuations, main values, and real value anomalies, there will be an increased standard distribution (Xu et al. 2014).

FCR cannot be utilized as a selection index to decide whether feed consumption or bodyweight gain is optimal, which decreases the variability of group selection and affects the selection performance (Yi et al. 2018). From a genetic point of view, FCR is a limited gain factor and is used as an indicator of the effects of other genetic development (O'Sullivan et al. 2019). This type of choice leads to the synchronous selection of feed intake or bodyweight gain with a population improvement bias towards high feed intake and high BWG.

2.4.0 Factors affecting FCR

2.4.1 Genetics/breed

In agriculture, a major concern is the effective and productive use of energy during production. Due to the increased use of farmland and plant products for ethanol and energy production over the last ten years, there is an economic strain on the production of animals due to higher feed costs (Popp et al. 2016). Feed conversion efficiency is a significant trait in breeding schemes since this is a possible solution to concerns of feeding costs as well as increasing productivity and reducing greenhouse gas emissions, according to studies of most livestock species (Bacon et al. 2012). Most performance-based traits show that recent attempts to increase feed conversion efficiency in broilers are mostly related to genetic selection (around 85-90 percent); feeding and management techniques account for only about 10-15 percent of phenotypic development (Alberti 2015). Furthermore, individual variations in energy requirements for growth, maintenance, and thermogenesis affect weight gain as an observable component of feed

efficiency (Swennen et al. 2004). As a result, a wide range of genes can have an impact on FCR. Genetic selected programs over the past 6 decades have resulted in rapid growth rates and improvement in meat yield among broilers, dramatically cutting down the age at slaughter and the amount of feed and energy necessary to raise these birds to market weight (Tallentire et al. 2016). Over the last six decades, growth rates have increased by over 400 percent (Zuidhof et al. 2014).

2.4.2 Quality of feed

Feed quality has a significant influence on the performance of chickens. It is, therefore, necessary to use adequate ingredients and processing methods to supply a suitable balanced diet that would contribute to optimum broilers' performance. After water, energy and protein are the second and third most important feed constituents for maintaining health, development, and productivity (Ravindran 2013c). Cereal grains produce 60-70 percent of chicken's daily nutrition, with the remainder coming from other energy and protein sources (Ahiwe et al. 2018). Feed ingredients such as fish meal and soybean meal are used as the primary protein sources in chicken diets (Ravindran 2013b). This is because they have a high protein content (Awachat et al. 2012; Brah et al. 2017). A series of studies have indicated that the supplementation of phytase in diets of broilers has a significant effect on growth performance, mineralization of bone, utilization of nutrients and minerals, and the overall welfare of birds (Olukosi et al. 2013). Feeding low phytase diets to birds for more than 48 hours could confound phosphorous (P) digestibility effects potentially due to physiological adaptations in birds (Li et al. 2015; Perryman et al. 2016). Adaptive changes may be stimulated in the gastrointestinal tracts (GIT) of birds to maintain P homeostasis. The highest impact of the digestibility of phosphorous and phytase efficacy occurred within two to five days as compared to long periods (16 days) when phosphorus-deficient diets

were fed to birds (Babatunde et al. 2019). When compared to broilers fed an arginine-deficient diet 90 percent NRC recommendation (Arginine: Lysine = 1.02 and 0.99 in starter and grower phases, respectively), broilers fed a diet with an arginine level of 100 percent NRC recommendation (total Arginine: Lysine = 1.14 and 1.10 in starter and grower phases, respectively) demonstrated lower FCR at 21 days, as well as from 1 to 42 days (Ebrahimi et al. 2014). In another study, no significant difference ($P > 0.05$) was observed in terms of FCR in broilers given diets with arginine levels either at the recommended 100% or exceeding (105 and 110%) the NRC recommendations (Laika and Jahanian 2017). A recent observation indicated that at a duration of 21 and 42 days, there was a quadratic increase in bodyweight when the birds were fed with dietary arginine supplemented diet, with the birds given an arginine-deficient feed (total Arginine: Lysine ratio = 0.67 (starter) and 0.69 (grower)) or the maximum arginine supplementation level (total Arginine: Lysine ratio = 2.07 (starter) and 2.53 (grower)) indicating a lower bodyweight when compared to the other treatments (Xu et al. 2018).

2.4.3 Growth Enhancers

The trend in chicken production is changing from birds per unit land area to meat production per unit land area and farmers usually stock more birds than standard to increase profit (Thomas et al. 2004; Estevez 2007). However, high-density stocking of poultry increases the likelihood of infectious disease transmission, and producers tend to use antibiotics to correct for increased stocking density. The effect of synbiotics (combination of *Bacillus subtilis* (probiotic) and mannan oligosaccharides (prebiotic)) was observed on chicken performance and showed that birds fed the basal (control, no growth promoters) diet showed the highest feed intake whereas the birds given symbiotic in the diet showed the least ($P = 0.0008$) feed intake (Altaf et al. 2019). They

reasoned that supplementation of growth promoters like synbiotics in broiler diets improves the biological functions of the essential microbes in the gut of the host chicken and elevates the nutrient absorption thereby reducing the feed intake. In addition, the synbiotic-fed chicken had improved ($P = 0.0001$) FCR compared to other treatment groups and it was indicated that the increased FCR in the synbiotic-fed population was due to the symbiotic-induced improvement in the intestinal environment tract (Altaf et al. 2019).

The impact of prebiotic, probiotic, and synbiotic supplementation on one-day-old chicks was investigated and it was established that the final bodyweight was significantly ($P < 0.05$) higher in the probiotic and synbiotic supplemented broilers compared to the control and prebiotic groups (Abdel-Raheem et al. 2012). The effects of probiotic (organic-green culture-zs) at levels of 0.1%, 0.2%, 0.3%, or 0.5% were studied and it was found that the bodyweight of chicken fed 0.2-0.5% of probiotics was significantly ($P = 0.034$) greater than chicken fed without the probiotic diet (Khan et al. 2011). The effects of probiotics *(*in this case *Saccharomyces cerevisiae)* and prebiotics (mannan-oligosaccharide) supplementation in the diet of broiler chickens was studied for six weeks and it was observed that the bodyweight improved significantly ($P < 0.05$) with dietary inclusion of the probiotic and the prebiotic compared to the control diet (no probiotic or prebiotic) (Shahir et al. 2014). Chickens supplemented with antibiotics, probiotics, prebiotics, and synbiotics had a lower feed conversion ratio than chickens in the control treatment ($P < 0.01$) (Ghahri et al. 2013). The effect of antibiotics (phosphomycin), probiotics, prebiotics, and synbiotic was compared on broiler chickens for 6 weeks and revealed that birds fed synbiotic had an improved ($P < 0.01$) feed intake as compared to those of other treatments (Ghahri et al. 2013). The impact of direct-fed microbial (DFM) was assessed for 35 days on chickens fed with basal diet only or basal diet mixed with either virginiamycin, as an antibiotic growth promoter or *Lactobacillus*

reuteri (DFM 1) or a mixture of *L. reuteri*, *B. subtilis*, and *S. cerevisiae* (DFM 2) (Salim et al. 2013). It was observed that the broiler bodyweight gain was significantly increased ($P < 0.05$) by dietary AGP and DFM supplementation from 0 to 21 days, but feed consumption and feed conversion ratio were significantly decreased ($P < 0.05$) when birds were fed DFM 2 from 0 to 7 days. A significant improvement in FCR in chickens fed different growth promoters such as synbiotics was observed compared to those fed with the control diet (Das et al. 2016).

2.4.4 Farm management practices

Broiler housing and its management attempt to ensure bird health and productivity (Mesa et al. 2017). Chicken housing design is critical for bird welfare, growth, development, and productivity. As a result, the system of poultry housing employed by the poultry farm is determined by the predominant climatic conditions in the area where the farm is situated. Although the controlled housing system is the most prevalent in temperate regions, the open poultry house system has been deemed a successful method of housing in tropical regions due to its ease of building, low management cost, and heat management (Oloyo 2018). The total heat produced in a poultry house encompasses the amount of heat produced by the birds, the surrounding atmosphere, and fecal material biodegradation (Clark 2013). As a result, the type of housing structure to be used in the poultry farm is a significant determinant element in the management tool to be used. In comparison to natural ventilation, mechanical ventilation is correlated with a higher FCR (Van Limbergen et al. 2020). This is mostly due to improved air quality in mechanically ventilated houses in general, as the developing chicken requires a lot of oxygen to maintain rapid growth and feed conversion efficiency.

Broiler stocking density has a significant impact on health and wellbeing, higher density stocking is often correlated with heat-related problems and infections (Abudabos et al. 2013). However, some findings have also indicated that increasing feeding by socially facilitated habits requires a certain level of bird density (Collins and Sumpter 2007). Studies indicate that stocking broilers from fourteen to eighteen chickens per meter square is optimal to ensure good animal productivity and profitability (Kryeziu et al. 2018).

To limit the risk of pathogens in broilers or pathogens of zoonotic concern, good biosecurity techniques are critical in production (Graham et al. 2008; Bojesen et al. 2003; Newell et al. 2011). In the case of hemolytic *Gallibacterium spp.*, lower levels of biosecurity contribute to a higher prevalence of disease, as well as a higher chance of flocks becoming infected with thermophilic *Salmonella spp.* or *Campylobacter spp.* (Gibbens et al. 2001; Liljebjelke et al. 2005; Osimani et al. 2017; Bojesen et al. 2003). The control of avian influenza (AI) also necessitates biosecurity (Graham et al. 2008; Conan et al. 2012). As a result, improved biosecurity on poultry farms and restrictions on live-bird migration are critical control measures included in most national AI eradication plans (Conan et al. 2012). Biosecurity activities in 80 commercial poultry farms in Nigeria were investigated using a biosecurity ranking method to rate farms based on biosecurity measures in their research. The researchers discovered a relationship between increased biosecurity practices and a decrease in disease outbreaks (Maduka et al. 2016). In addition, the study also concluded that increased biosecurity practices would benefit chicken productivity and that knowledge sharing and awareness programs could improve practices (Maduka et al. 2016). A mixed-method approach that included direct findings/observations, a questionnaire survey, and interviews with 463 poultry supply chain stakeholders was employed by (Negro-Calduch et al. 2013). The information was gathered to determine small-scale broiler producers' biosecurity

activities and measures in central Egypt, which could help researchers better understand the factors that contribute to disease transmission within and between farms. The findings revealed that a number of critical biosecurity policies were rarely applied by the producers. The authors listed that vaccinators, as well as all other staff, should practice personal hygiene, changing clothing, and disinfecting during farm visits as best practices for improving biosecurity (Negro-Calduch et al. 2013). A comprehensive analysis of the biosecurity practices structure in Dutch poultry farming to assess the activities and risks associated with the introduction of viruses and infectious diseases into poultry farms was conducted by (Ssematimba et al. 2013). Their findings revealed that bird-to-bird interaction during flock thinning, and restocking processes is the riskiest contact form. Human-to-bird interaction while entering poultry houses was also discovered to be a significant source of risk (Ssematimba et al. 2013).

The addition of a lighting device to a bird's environment at a young age has little or no impact on the hormonal system; instead, it promotes the activeness of birds, which includes growth, feed consumption, physical, and physiological activities (Mendes et al. 2013). However, increased lighting durations and intensity can result in fatigue, immune responses, leg anomalies, cannibalism, and even mortality (Mendes et al. 2013). The continuous lighting schedule of 16 hours of light and 8 hours of darkness is the most widely used lighting program, and it has proved to be effective in terms of overall chicken performance (Clark 2013). When compared to birds reared under red and orange light, birds reared under light sources which are yellow, green, and blue have been found to have increased bodyweight (Jiang et al. 2012; Kim et al. 2012). Birds produced under blue light exhibit docile characteristics, while those produced under red light exhibit more active and aggressive behaviours (Lewis and Morris 2000). In addition, the red light was shown to increase bird sexual behaviour. Farms that could adapt to the intensity of light in the

broiler house had a lower FCR (Van Limbergen et al. 2020). Farms with the ability to adjust the light intensity in the broiler house may use this technique in their management to promote or slow down broiler feed intake, as expected by breed requirements, and thus increase broiler performance characteristics such as FCR (Van Limbergen et al. 2020).

To satisfy their everyday nutritional requirements, broilers need access to feed. For productive and welfare-oriented broiler processing, adequate feeder space that enables birds to eat at their leisure is critical. Inadequate feeder space will contribute to aggression, competition, frustration, and poor welfare of chickens (Sirovnik et al. 2018), whereas too much feeder space contributes to inadequate utilization of resources in chickens (Oliveira et al. 2019). Researchers can now monitor the feeding period, frequency of feeder visits, and position of individual birds in small-scale pens using precision agricultural instruments (Li et al. 2019; Oliveira et al. 2019). There is a strong preference for broiler chickens when it comes to the location of feeders (Li et al. 2020), and many birds can share the same feeders when the location of the feeder is at their desired location. Not only should the feeder allowance be addressed, but also the correct configuration of the feeder allowance, ensures the birds eat at their own will.

Broilers favour bell drinkers or troughs over nipple drinkers when given a preference, in order to exhibit their stereotypic "scoop" behaviour when drinking water (Houldcroft et al. 2007). Nipple drinkers have a significant benefit in that less water is leaked. A trend toward a better FCR was observed in flocks where farmers tested the flow rate of the drinking system for abnormal variability. When compared to feed and water *ad libitum* treatments, lack of water had the same effect as a lack of feed, causing a higher number of villi per area and a decrease in the size of the villus, resulting in inefficient nutrient absorption and higher FCR (Huang et al. 2011).

2.4.5 Environmental conditions

Hereditary factors play a role in chicken growth performance, but environmental factors have a significant impact as well (Babinszky et al. 2011). The nutritional efficiency of chickens is affected by a number of factors, including environmental conditions such as stocking density and climate (Gholami et al. 2020). In a study of Arbour Acres and Hubbard strains in a warm environment, bodyweight gain, feed conversion ratio, carcass consistency, and meat quality were observed to be significantly chicks ($P < 0.5$) higher in Arbour Acres than Hubbard (Attia et al. 2016). Providing optimal growth conditions for birds in different environments can be a cost-effective way to improve their health and efficiency (Costantino et al. 2018). In comparison to alpine climates, it was found that warm and dry weather conditions, as well as temperate and humid climates, have improved effects on chicken growth performance such as bodyweight gain and feed conversion ratio (Bouyeh et al. 2017).

There is a substantial impact of temperature on feed consumption during the starter, grower, and entire experimental durations ($P < 0.05$) (Gholami et al. 2020). When chickens are exposed to high temperatures (>32^{o}C) in their habitat, they exhibit a variety of behavioural and physiological changes that enable them to re-establish heat equilibrium with their surroundings. Chickens eat less and spend more time drinking and panting as the temperature rises (Mack et al. 2013). High ambient temperatures can be unpleasant for commercial broilers, and when combined with high humidity, they can suffer much more damage. Seasonal fluctuations have a significant impact on the comfort of broilers, lowering growth rate, feed conversion, live weight gain, and overall productivity (Ahaotu et al. 2019).

When birds are subjected to high temperatures in the field, they develop physiological, behavioural, and immunological responses that negatively impact their growth performance and

productivity. As a result of stunted growth, decreased hen-day yield, higher production costs, and higher mortality due to lowered immunity and reproductive failure, a hot climate can have a serious effect on poultry output, resulting in significant economic losses in poultry production (Ahaotu et al. 2017; Ononiwu et al. 2017; Nkwocha et al. 2018).

Broiler chickens fed enriched diets in a regulated warm environment improved growth performance, liver weight, and blood parameters but increased abdominal fat (Awad et al. 2017). Under heat stress and high stocking density, chickens ingest less feed, and extreme feed deficiency results in reduced absorption of amino acids and other vital nutrients, resulting in decreased productivity (Ike 2011). When broilers are subject to heat stress, certain alterations in the blood supply occur (disruption of acid-base equilibrium, elevated blood pH, and respiratory alkalosis), and the cardiovascular system is one of the mechanisms that interfere with heat dissipation, which can influence the amount of albumin (Zhang 2015). Reduced feed intake is one of the most serious issues in a dry environment. This, in particular, inhibits body growth and development, resulting in a weakened immune system as a result of the dry climate's negative chain reaction (Amini et al. 2015). In a research conducted in both the tropics and temperate areas, broilers in the temperate zones had a significantly ($P = 0.04$) lower feed consumption than those in the tropical and subtropical zones (Osti et al. 2017). When they compared the relationship between climatic zones and seasons, they discovered that broilers produced in the temperate zone during the winter had a significantly ($P = 0.031$) lower feed consumption than those produced during the summer. Also, broilers raised in tropical and subtropical areas had significantly ($P = 0.021$) higher bodyweight than those raised in temperate zones, according to (Osti et al. 2017). In terms of FCR, a significant ($P = 0.004$) impact of seasons on feed conversion ratio was reported, with the better FCR achieved

during the winter compared to summer, and in terms of climatic zones, the best FCR (P = 0.025) was achieved in the subtropical zone (Osti et al. 2017).

2.4.6 Infectious Disease

The poultry industry has made significant progress in terms of enhancing bird performance and sustainability. However, the disparity between the birds' ability and their current performance in real-world situations is widening due to issues related to animal welfare and sustainability (Hans-Wilhelm, 2017). In conventional broilers, a significant number of infectious and non-infectious risk factors have already been reported as contributing to lower production and high mortality (Jones et al. 2019). For example, Avian Pathogenic *Escherichia coli* (APEC) is known to cause disease and mortality in broilers (Fancher et al. 2020; Kim et al. 2020). APEC is the most common cause of neonatal septicaemia, which leads to high mortality rates (Revitt-Mills et al. 2015). Antibiotic resistance is also linked to the presence of APEC (Kunert Filho et al. 2015). Stress, which can be caused by a variety of poor husbandry activities, is the most significant predisposing factor for APEC infections in broilers (Poulsen et al. 2020).

Skin lesions were found to be a significant cause for the destruction of poultry carcasses in French slaughterhouses in a recent survey (Salines et al. 2017; Lupo et al. 2008). Some infectious diseases can be prevented by ensuring that adequate facilities are provided, for example, cracks in a poultry house's concrete floor create an area that can't be adequately washed and disinfected between flocks, allowing various pathogens to propagate and cause disease in subsequent flocks (Daehre et al. 2018).

Flocks having problems with necrotic enteritis had slightly higher FCR (Van Limbergen et al. 2020). Researchers have previously shown that necrotic enteritis may include chronic intestinal

mucosal injury, which contributes to poor feed utilization and an increase in FCR (Timbermont et al. 2011; Paiva and McElroy 2014). Farmers who tested the flow rate of the drinking system in case of abnormal variations saw a trend toward a better FCR in their flocks (Van Limbergen et al. 2020). This is because water plays an important role in all metabolism activities and ensures maintenance of the bird's homeostasis, digestion, and elimination of waste. Broilers consume almost twice the amount of water by weight compared to feed intake (Lacy 2002). This means adequate water flow rate (ml/min) (estimated by 7ml x the age of the birds (weeks) + 20) (Lott 2003), ensures more water availability to the birds which will aid in the digestion of ingested feed and hence, better FCR.

Coccidiosis occurs when protozoan parasites of the genus *Eimeria* intensively invade and cause tissue damage in the intestine, resulting in disruption of digestion, absorption, and assimilation of nutrients, high chances of infestations by other disease agents, slow growth rate, and death in extreme cases (McDougald 1998; Williams 2005). The prevalence of coccidiosis problems was clearly seen to be a risk factor in Yegani and Korver's model for regular growth in broilers (Yegani and Korver 2008). Coccidiosis has been found by several researchers to have a detrimental impact on health. *Eimeria spp.* colonize the small intestine and, due to their lifecycle, inflict significant harm to intestinal epithelial cells, resulting in intestine dysfunction and decreased daily growth. Farms with a history of dysbacteriosis issues have a lower European Broiler Index (EBI = (Average grams gained/day X % survival rate) X 100/Feed Conversion X 10)) (Marcu et al. 2013). This index compares and standardizes the technical results from broiler production, which includes mortality, feed conversion, and daily weight gain. This is mostly due to dysbacteriosis' negative impact on everyday growth and FCR, which has pathophysiological

consequences similar to those mentioned above for chronic necrotic enteritis' impact on FCR (Yegani and Korver 2008).

2.5 Antibiotics / Antimicrobial Growth Promoters (AGPs)

Antibiotics are used to combat bacterial infections in animals and humans. The invention of antibiotics was a breakthrough in managing infectious diseases and enhancing feed efficiencies in the agricultural industry (Mehdi et al. 2018; Engberg et al. 2000). Antimicrobials can treat a variety of pathologies, specifically, antivirals treat viral infections, antifungals can treat fungal infections, and the term antibiotics generally refers to drugs that treat bacterial infections. Antibiotics can be used as prevention or curative care in phytosanitary procedures, fish farming, animal nutrition, and human or veterinary medicine. The use of AGPs has contributed greatly to increased agricultural productivity, by reducing mortality rates and increasing growth rates by removing problematic bacteria (Mehdi et al. 2018; Diarra and Malouin 2014). However, projections now show that AMR will result in the deaths of 10 million people annually if steps are not taken to reduce the use and misuse of antibiotics (Sugden et al. 2016). Agricultural use of antibiotics is particularly problematic since antimicrobials are often administered through feed, directly targeting the GIT, and in low dosages that are used foster the evolution of resistance (You and Silbergeld 2014).

Antibiotics such as tetracycline, penicillin, macrolide, sulfonamide, aminoglycoside, and cephalosporin are commonly used in livestock production, particularly in North America (https://www.mordorintelligence.com/industry-reports/north-america-feed-antibiotics-market-industry). Tetracyclines represent about 37% of antibiotics given to animals in the European Union (EU) (Carvalho and Santos 2016; Ronquillo and Hernandez 2017), whiles they represent about 71% in the US (Ronquillo and Hernandez 2017). According to (ESVAC 2019), the use of

antibiotics as growth promoters in animal production is banned in countries with the European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) membership.

2.6 Antibiotic resistance and its effects on animals, humans, and the environment

Current animal production relies on the routine use of antimicrobials; therefore, raising the burden of selection on bacteria to become resistant (Van Boeckel et al. 2015). Per year, about 700,000 people die from infections caused by multi-resistant bacteria in Europe, according to the European Centre for Disease Prevention and Control (ECDC), and these microorganisms cost Europe around 1.5 billion euros in increased healthcare facilities and lower productivity (Carlet and Mainardi 2012). The United States, in addition, spends about 35 billion USD annually according to a report by the World Health Organization (WHO) (Leung et al. 2011). AMR bacteria of animal origin can be transferred to humans via the environment (Graham et al. 2009) and food items (Price et al. 2005) and to farmworkers through direct interaction (Smith et al. 2013). The misuse of antibiotics has resulted in drug residues in animal products in addition to bio-resistance (Ronquillo and Hernandez 2017).

The contamination rates of priority bacteria transmitted by farm animals have been reported (Table 1). This was done by monitoring the transmission of the bacteria through animals using the Public Health Agency of Canada's (PHAC) Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) system.

Table 3: Contamination rates of priority bacteria transmitted by farm animals.

Priority Bacteria	Farm Animal	Contamination rate (%)
Salmonella Spp.	Chicken	34
	Pork	16
E. coli	Chicken	96
	Beef	56
	Pork	55
Campylobacter	Chicken	25
	Beef	87
	Pork	73

Source: CARSS (2016)

Antibiotic resistance has been linked not only to human infections but also to livestock infections such as porcine reproductive and respiratory syndrome (PRRS) (Chung et al. 1997) and extraintestinal pathogenic *Escherichia coli* (ExPEC) strains causing economic losses in the chicken industry (Mellata 2013). Given that chickens raised intensively can serve as a reservoir for AMR in animals, there is the need to assess its effects on humans, the environment, and other animals (Hedman et al. 2020). Other adverse effects that could outweigh the long-term advantages of improved productivity of antibiotics usage in poorly controlled intensive farming include reduced nutritional quality of meat (Sami et al. 2004), soil and water pollution (Gerber et al. 2005), and biodiversity loss (Tscharntke et al. 2005).

The use of antibiotics in animal feed has been banned in many countries because of the rising levels of AMR; Sweden was the first to ban AGPs in 1986, followed by Denmark in 1998 and there was a total ban on the use of AGPs by the EU in 2006 (Castanon 2007). Even though the

EU has banned antibiotics used for growth promotion, the coverage of the ban was limited to antibiotics which is important in human medicine such as vancomycin which causes vancomycin-resistant enterococci (VRE) in humans (Health and Services 2013; Wielinga et al. 2014; Wegener et al. 1999). The ban on the subtherapeutic use of antibiotics in the diets of animals has contributed to the reduction in animal productivity (Cheng et al. 2014). This is partially because of increased rates of livestock infections (Hao et al. 2014). Meanwhile, there was an increase in the overall quantity of antibiotics used in animals as the use of therapeutic antibiotics and disinfectants increased dramatically due to the high occurrence of diseases arising from the ban. The discovery and production of new antibiotics have declined significantly for decades (Stanton 2013). From 2006 to 2010, there was an increased antimicrobial shortage by 283% mainly as a result of decreased supply of antibiotics by manufacturers due to legal regulations and/or increased demand when there was an announcement of new therapeutic indications for a drug (Borchardt and Rolston 2013). Globally, intensive livestock processing has expanded food production at a low cost per unit produced, but maybe at an unrecognized price compensated for by increasing resistance to antimicrobials (Van Boeckel et al. 2015).

2.7 Eubiotics as an alternative for AGPs

Researchers are interested in using alternatives to antibiotics for growth promotion. A variety of alternatives/replacements to antibiotics have been proposed to overcome the elevated mortality and morbidity rate attributed to the restriction of in-feed antibiotics (Cheng et al. 2014; Millet and Maertens 2011; Seal et al. 2013). These products have many benefits over commercial antibiotics that are widely used because they are residue-free and universally accepted as healthy and commonly used products in the food industry (Varel 2002). Some of these alternatives are

probiotics, prebiotics, synbiotics, organic acids, essential oil compounds, phytogenic feed additives (PFAs), enzymes and amino acids, etc.

2.7.1 Probiotics

The WHO defined probiotics as “live microorganisms when administered in adequate doses, contributes to the beneficial health state of the host” (WHO, 2001). Various microorganisms from bacteria, fungi and yeast origin are used in the preparation of probiotics for poultry (Popova 2017). Over the last decade, the use of probiotics as feed supplements in animal production has significantly increased (Mingmongkolchai and Panbangred 2018). The mechanism of action of probiotics in livestock production system includes generating and maintaining healthy gut microflora, improving digestion and nutrients utilization, competitive exclusion of harmful bacteria, decreasing pH and release of various substances which are antibacterial in nature, toxin neutralization, competition with pathogens for nutrients, reduction in ammonia production, and stimulation of the immune (Callaway et al. 2008; Dhama et al. 2011; La Ragione et al. 2004). Probiotics can also create antimicrobial compounds, modify immunological responses, and affect the gut's metabolic activity (Kosin and Rakshit 2006).

Probiotic feeding has the potential to promote gut health while also increasing feed efficiency during the growth stage (Gao et al. 2017). It has been shown that effective probiotics help to improve the development of the microflora in chicks and poults (Bansal et al. 2011). As feed additives, probiotics (*B. subtilis* and *E. faecium*) showed a positive impact on the poultry performance by offering digestible proteins, enzymes, vitamins, and other important co-factors and by decreasing gut pH through the production of lactic acids. Further, *B. subtilis* and *E. faecium*

help in the metabolism of minerals and the synthesis of vitamins including Biotin, and vitamins B1, B2, B12, and K which are responsible for proper growth and metabolism (Hatab et al. 2016).

The use of probiotics such as *E. faecium, Bifidobacterium animalis, Lactobacillus reuteri* and *B. subtilis* in feed also had coccidiostatic action against *Eimeria tenella*. A reduction of the impact of parasitic infection on chickens in the absence of anticoccidial infections using these probiotics as treatment was observed preserving the intestinal health of chickens (Giannenas et al. 2012). Recent studies have demonstrated an antibacterial effect on bacterial microflora in the small intestine of chickens using probiotics such as *E. faecium* (Levkut et al. 2012), *B. subtilis* (Zhang et al. 2013), and *Streptomyces sp.* (Latha et al. 2016), in the diets of chicken. The highest level of growth performance and immune response in broiler chickens was induced by feeding probiotics (*Lactobacillus plantarum* strain LP-8) when compared to antibiotics (chlortetracycline and salinomycin) and the control diet (Gao et al. 2017).

2.7.2 Prebiotics

Prebiotics are non-digestible feed additives that have a positive impact on the health of the host due to their fermentable properties that promote the production and/or activity of bacteria in the ileum and caecum (Gibson and Roberfroid 1995). Prebiotics are a source of carbon and energy for the probiotic strains of bacteria already inhabiting the colon, where bacterial fermentation processes of some nutrients occur (Dankowiakowska et al. 2013). The effects of dietary supplementation with the prebiotics fructooligosaccharides (FOS) and mannan-oligosaccharide (MOS) for 4 weeks were investigated in broiler chickens (Ross) and the results showed no significant differences among the control and the supplemented groups in the overall feed intake, feed conversion, and mortality (Kim et al. 2011). The effect of prebiotics and AGPs on 90 unsexed

broilers (Cobb) was compared for 42 days and it was observed that the group supplemented with prebiotics showed a significant ($P < 0.05$) improvement in growth performance in comparison to the control and AGP-supplemented group (Helal et al. 2015). Different doses of prebiotics (0.1% MOS, 0.2% MOS, 0.1% FOS and 0.2% FOS) were tested against BMD and a control diet and it was observed that dietary supplementation of the prebiotics (especially 0.2% MOS) had significant ($P < 0.05$) improvement in the bodyweight gain of broiler chickens (Biswas et al. 2019). The use of prebiotics in broiler feeds could effectively and economically replace AGP in the promotion of growth.

2.7.3 Phytogenic feed additives and Essential Oils

Phytogenic feed additives can be used as growth promoters and are obtained from plants, herbs, and spices. Most PFAs have been used in human nutrition for years as food preservatives, flavours, and medicines either in solid, dried, or ground forms or as essential oils or extracts (Guo et al. 2003; Applegate et al. 2010). The successful use of PFAs as an alternative to antibiotics results from improved immunity, reduced stress response, and their positive effects on growth in animal production (Mehdi et al. 2018). Most phytogenics are widely available and contain more chemical substances with an antioxidant, sedative, antidepressant, antiviral and bactericidal properties making them suitable to be utilized as feed additives for growth promotion (Duke and Beckstrom-Sternberg 1994; Applegate et al. 2010).

Essential oils are the hydrophobic solvent of a plant's odoriferous and volatile aromatic compounds. Natural (vegetable origin) or synthetic oils may be classified as essential oils. Only a few essential oils have useful antibacterial properties. Thymol, transcinnamaldhyde, carvacrol, and eugenol are among the most commonly used (Mehdi et al. 2018). Essential oils are extracted from

plant materials and are complex mixtures of low-boiling-phenylpropenes and terpenes of secondary plant metabolites. The characteristic plant essences and fragrances are specifically associated with essential oils (Greathead 2003). Their activity is based on their interference with the bacterial enzymatic mechanism and the regulation of immune responses and inflammation (Mehdi et al. 2018). Essential oils in chicken feeds have shown a positive result on the health, the quality of meat and carcass, and growth of chicken. Essential oils have been reported by experimental studies as a potent growth promoter based on their preventive and curative effect in necrotic enteritis (Jerzsele et al. 2012), anti-inflammatory effects, antimicrobial and antioxidant properties, their positive effects on lipid metabolism and digestion stimulation properties (Acamovic and Brooker 2005; Brenes and Roura 2010). It has been reported that peppermint (*Mentha piperita*) is a good alternative to the conventional antibiotic (virginiamycin) in broilers (Emami et al. 2012). Increased average daily gain (ADG), improved thigh muscle percentage, and reduced abdominal fat percentage were reported in broilers when they were fed diets containing oregano essential oil (*Origanum genus*) at an inclusion rate of 300 and 600 mg/kg diet (Peng et al. 2016).

2.7.4 Amino acids and enzymes

Enzymes used as feed additives are produced through the fermentation of products from fungi and bacteria. These enzymes are basically added to animal feed to maximize feed conversion by facilitating the degradation of components such as proteins, glucans, and phytates (Mehdi et al. 2018).. Commercially available enzyme feed additives are classified into four categories: (1) microbial phytases, (2) viscous cereal glycanases, such as wheat and barley, (3) non-viscous cereal enzymes, such as corn and sorghum, and (4) non-cereal enzymes, such as soybean meal and grain

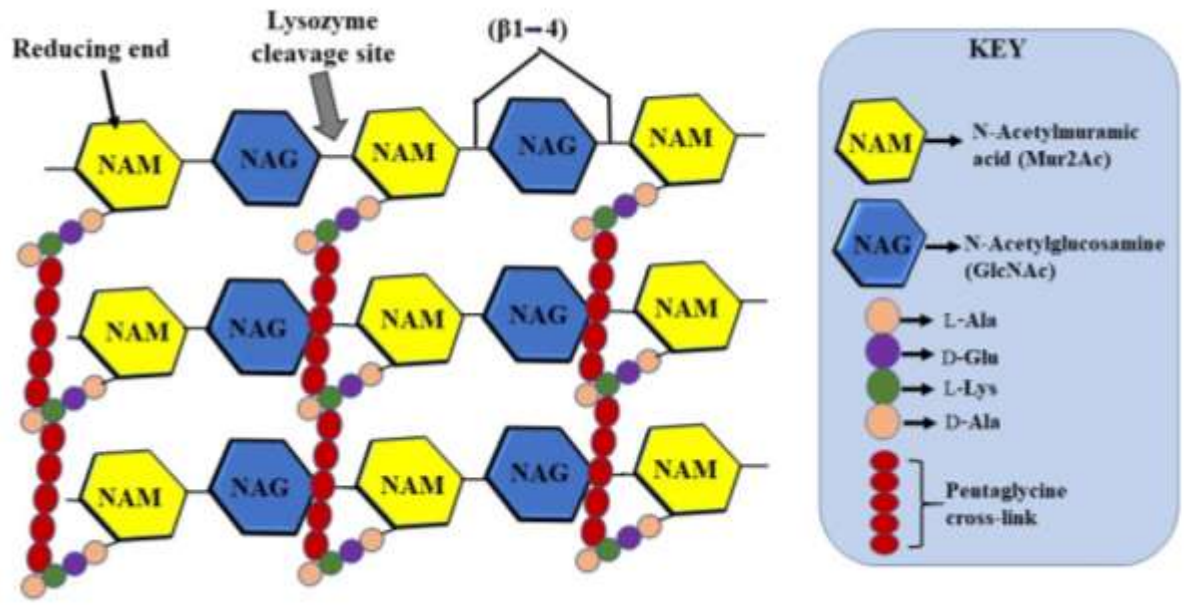
legumes (Ravindran 2013). Synergistic effects on nutrient utilization and animal performance may be achieved when enzymes are used in combination (Cowieson and Adeola 2005).

Lysins are bacteriophage endolysins that provide an innovative antibacterial therapeutic choice. When added exogenously to Gram-positive bacteria lysins can induce bacterial cell lysis (Fenton et al. 2010; Rios et al. 2016). In poultry, a mixture of lysins including peptidases, amidases, and lysozymes has antimicrobial activity against *C. perfringens* (Volozhantsev et al. 2011). Ply3626 lysine, for example, is an enzyme that has been proven to have lytic action against many strains of *C. perfringens* (Fenton et al. 2010; Zimmer et al. 2002).

Phytase enzyme can increase villus width and reduce crypt depth which can lead to the improvement of ADG in broilers (Mohammadagheri et al. 2016). Digestion of feed was improved when endo-b-1-4-xylanases and b-1-3,1-4-glucanases were used in wheat and barley feed of broilers (Cowieson et al. 2006).

Lysozyme (EC 3.3.1.17) a peptidoglycan hydrolase, also called muramidase or N-acetylmuramide glycanhydrolase, is a glycoside hydrolase that is a natural antimicrobial protein, and it is considered as a vital component of the body's innate immune system (Liu et al. 2010). It can act as a bacteriolytic agent by hydrolyzing the -1,4-glycosidic bond between N-acetylmuramic acid and N-acetylglucosamine in the bacterial cell wall, which is especially effective against Gram-positive bacteria (Ibrahim et al. 1994). Lysozymes can be found in secretions such as saliva, tears, human milk, mucus, and large quantities can be found in egg white. Lysozyme is commercially derived from eggs due to its availability of egg white and has been used to preserve foods naturally and is a medicinal drug for humans (Sava 1996). In addition, lysozyme administered intraperitoneally in mice by *in vivo* method reduces the pathology caused by *Klebsiella pneumoniae* (Ivanovska et al. 1996). This means that lysozymes may serve as protection against

bacterial infections. Exogenous lysozyme's antimicrobial activity against *C. perfringens* type A in broiler chickens was related to necrotic enteritis, according to findings from in vitro studies. Lysozyme was shown to have the capacity to regulate *C. perfringens* type A (Zhang et al. 2006; Zhang et al. 2010). In comparison, when chickens were experimentally infected with *C. perfringens* orally, exogenous lysozyme decreased the amount of *C. perfringens* in the ileum and prevented lesions of the intestines (Liu et al. 2010). Anti-nutritional factors in feeds that modulate microbiota and immune function, enhance intestinal morphology, and reduce gut oxidative stress may be minimized by changes in the microbiota (Lallès 2016). A recent study indicated a significant ($P < 0.05$) improvement in bodyweight gain (BWG), FCR, the European production efficiency factor (EPEF), and EBI when broiler chickens were fed with diets supplemented with lysozyme, especially in the LYZ90 group (Abdel-Latif et al. 2017). In addition, the supplementation of dietary lysozyme (concentrated (≥90%, ≥40,000 units/mg protein) lysozyme powder from chicken egg white (Sigma)) or antibiotic (Flavomycin) in the diets of broiler chickens had no significant effects on the growth performance of broiler chickens (Xia et al. 2019). The effect of lysozyme on growth efficiency may be attributed to improved gut antioxidant and immune genes, as well as a substantial increase in intestinal villi, which increases the intestinal surface area available for absorption of nutrients (Humphrey et al. 2002). Higher doses of lysozyme can suppress *Lactobacillus* growth, which can boost broiler chicken growth performance (Jin et al. 1998) by stimulating enterocyte growth with an increase in intestinal villi and crypts, which favours digested nutrient absorption (Pan and Yu 2014).



Adapted from: (Proteomics (n.d))

Figure 1: The network structure of a peptidoglycan.

CHAPTER 3. MATERIALS AND METHODS

3.1 Ethics Statement

All procedures and experiments in this research were carried out in full compliance with the Faculty of Agricultural and Environmental Sciences (FAES) Animal Care Committee (ACC) guidelines. All procedures were approved by the FAES ACC.

3.2 Study site, source of feed, and duration of study

The feeding trial was undertaken at the Donald McQueen Shaver Poultry Complex, Macdonald Campus, McGill University, Canada. The base feed (starter and grower) used in this experiment was obtained from Belisle Solution Nutrition Inc. (St-Mathias-sur-Richelieu, Qc), with a special production that was free from any traces of additives. Corn and soybean were used as the primary ingredients in the feeds. Antibiotics, ionophores, coccidiostats, mycotoxin binding agents, or any growth-promoting supplements were not used in the feed. Phytase with an adequate matrix for calcium and usable phosphorus was also used in the diets (Table 2). The feeding trial lasted for 42 days (from March 16, 2020, to April 27, 2020).

3.3 Dietary treatments, Experimental Chicken, and Experimental Design

Birds were fed in 2 phases: starter (0-14 d) (23.0% CP and 2977 Kcal/kg diet) and grower (14-42 d) (20.1% CP and 3056 Kcal/kg diet) (Table 2) which meets the requirements of broiler chickens for respective phases according to the recommendations from the National Research Council (NRC) (NRC 1994). Diets were free of antibiotics and supplements and contained a commercial phytase. Diets were formulated using corn and soybean as the main ingredients to meet or exceed the nutritional recommendations of the broiler breeding company. Diets were free of antibiotics, ionophores, coccidiostats, mycotoxin binding agents, or any growth-promoting supplements. Diets

also contained phytase at 500 FTU/kg with an appropriate matrix for calcium and available phosphorus. All diets were formulated to be isonitrogenous and isocaloric. The results from the nutrient composition analysis were provided by Belisle and shown in Table 3.

Table 2: Formulation of the experimental starter and grower diets.

Ingredient	**Starter (D1 to 14*)**		**Grower (D14* to 42)**	
	Amount (kg)	**%**	**Amount (kg)**	**%**
Corn	547.36	54.74	532.85	53.29
Wheat	0	0	100	10.00
Soybean Meal (48% CP)	385.47	38.55	308.44	30.84
Soybean oil	21.59	2.16	22.5	2.25
Phosphorus	17.44	1.74	9.27	0.93
Calcium	15.39	1.54	16.16	1.62
Vitamin-Mineral Premix	5	0.50	4	0.40
Salt	2.73	0.27	3.61	0.36
Lysine HCl	1.2894	0.13	0	0.00
Methionine	1.4144	0.14	1.15	0.12
Threonine	0.2971	0.03	0	0.00
Calcium Chloride	1	0.10	1	0.10
Sodium Chloride	1	0.10	1	0.10
Phytase (25,000U/g)	0.02	0.00	0.02	0.00
Total	1000.00	100%	1000.00	100%

*Day 14 marks the end of the starter phase and the beginning of the grower phase (i.e. transional phase).

Table 3: Feed Composition Analysis[1].

Item	Starter (D1 to 14*)	Grower (D14* to 42)
ME, kcal/kg	2,977	3,056
Crude protein, %	23.00	20.1147
Lysine Total, %	1.43	1.11
Lysine Digestible, %	1.28	0.99
Methionine Total, %	0.51	0.44
Methionine Digestible, %	0.48	0.42
Cysteine Total, %	0.38	0.35
Cysteine Digestible, %	0.31	0.29
Threonine Total, %	0.94	0.78
Threonine Digestible, %	0.82	0.69
Tryptophan Total, %	0.29	0.26
Tryptophan Digestible, %	0.24	0.21
Arginine Total, %	1.61	1.40
Arginine Digestible, %	1.49	1.29
Histidine Total, %	0.62	0.55
Histidine Digestible, %	0.55	0.49
Isoleucine Total, %	1.09	0.96
Isoleucine Digestible, %	0.99	0.87
Leucine Total, %	2.01	1.81
Leucine Digestible, %	1.85	1.66
Phenylalanine Total, %	1.14	1.03
Phenylalanine Digestible, %	1.05	0.94
Tyrosine Total, %	0.92	0.83
Tyrosine Digestible, %	0.75	0.64
Valine Total, %	1.18	1.05
Valine Digestible, %	1.01	0.90
Crude Fat, %	4.45	4.60
Calcium, %	1.05	0.92
Phosphorus Total, %	0.75	0.56
Phosphorus Digestible, %	0.50	0.33
Sodium, %	0.22	0.24
Chloride, %	0.21	0.26
Potassium, %	0.93	0.82
Magnesium, %	0.19	0.18
Sulphur, %	0.27	0.25
Copper Total, mg/kg	28.11	26.93
Iron Total, mg/kg	266.96	180.89
Manganese Total, %	127.05	128.90
Selenium Total, mg/kg	0.50	0.51
Zinc Total, mg/kg	139.31	137.63
Cobalt Total, mg/kg	0.46	0.46
Fluoride Total, mg/kg	26.21	13.94
Iodine Total, mg/kg	0.80	0.80
Vitamin A Total, KIU/kg	6.41	6.43
Vitamin D Total, KIU/kg	3.00	3.00
Vitamin E Total, IU/kg	52.61	33.38

[1]Results provided by Belisle Solution Inc. (St-Mathias-sur-Richelieu, QC).

*Day 14 marks the end of the starter phase and the beginning of the grower phase (i.e. transional phase).

A total of 180 one-day Ross 308 male chicks were procured from a commercial hatchery. The chickens were vaccinated with Coccivac® - B52 (Merk Animal Health, Kirkland, QC, Canada) at the hatchery. All birds were housed together in a single floor pen (SFP) for fourteen (14) days and fed with a starter diet (Table 2 and 3) (starter phase). After day 14, the birds were weighed individually and the 160 birds closest to the mean bodyweight of the single pen were selected and randomly assigned to treatment pens (80 rooster cages, which had been modified for broilers) containing two birds per cage.

The selected birds were alloted to eight dietary treatments T1, T2, T3, T4, T5, T6, T7, and T8 (Table 4). Ten pens were assigned to each of the eight treatments. There were eight cages (pens) per block (housing unit), with one cage per treatment within each block; thus giving a Randomized Complete Block Design (RCBD). Within each block, there was a random allocation of the cages to the treatments (Fig.1). Each cage had two birds which represent a treatment within the block (housing unit). The figure (Fig. 1) below shows how the treatments were blocked:

	Cage 1	Cage 2	Cage 3	Cage 4		Cage 9	Cage 10	Cage 11	Cage 12
Unit 1	MYCHI	POCCH	BMD	Negative	Unit 2	NEOFI	TALPR	PENCH	MYCHI
	RHISO	NEOFI	TALPR	PENCH		POCCH	BMD	Negative	RHISO
	Cage 5	Cage 6	Cage 7	Cage 8		Cage 13	Cage 14	Cage 15	Cage 16
	Cage 17	Cage 18	Cage 19	Cage 20		Cage 25	Cage 26	Cage 27	Cage 28
Unit 3	TALPR	PENCH	MYCHI	POCCH	Unit 4	BMD	Negative	RHISO	NEOFI
	BMD	Negative	RHISO	NEOFI		TALPR	PENCH	MYCHI	POCCH
	Cage 21	Cage 22	Cage 23	Cage 24		Cage 29	Cage 30	Cage 31	Cage 32
	Cage 33	Cage 34	Cage 35	Cage 36		Cage 41	Cage 42	Cage 43	Cage 44
Unit 5	RHISO	NEOFI	TALPR	PENCH	Unit 6	POCCH	BMD	Negative	RHISO
	MYCHI	POCCH	BMD	Negative		NEOFI	TALPR	PENCH	MYCHI
	Cage 37	Cage 38	Cage 39	Cage 40		Cage 45	Cage 46	Cage 47	Cage 48
	Cage 49	Cage 50	Cage 51	Cage 52		Cage 57	Cage 58	Cage 59	Cage 60
Unit 7	PENCH	MYCHI	POCCH	BMD	Unit 8	Negative	RHISO	NEOFI	TALPR
	Negative	RHISO	NEOFI	TALPR		PENCH	MYCHI	POCCH	BMD
	Cage 53	Cage 54	Cage 55	Cage 56		Cage 61	Cage 62	Cage 63	Cage 64
	Cage 65	Cage 66	Cage 67	Cage 68		Cage 73	Cage 74	Cage 75	Cage 76
Unit 9	POCCH	NEOFI	PENCH	RHISO	Unit 10	BMD	MYCHI	Negative	TALPR
	Negative	TALPR	BMD	MYCHI		PENCH	RHISO	NEOFI	POCCH
	Cage 69	Cage 70	Cage 71	Cage 72		Cage 77	Cage 78	Cage 79	Cage 80

Figure 2: The layout of experimental treatments and cages.

The doses of each treatment are shown in Table 4.

Table 4: Treatment and doses of the various treatments.

Code	Treatment	Dose, mg/kg feed	Treatment duration, Day	No. of pens	Animals /pen	Total animals
T1	Control[1]	0	28	10	2	20
T2	LYZ25A_RHISO	30	28	10	2	20
T3	LYZ25A_NEOFI	30	28	10	2	20
T4	LYZ25A_TALPR	30	28	10	2	20
T5	LYZ25A_PENCH	30	28	10	2	20
T6	LYZ25A_MYCHI	30	28	10	2	20
T7	LYZ25A_POCCH	30	28	10	2	20
T8	BMD[2]	55	28	10	2	20
Total				80		160

[1]Diet was free from antibiotics, coccidiostats, ionophores, or any other feed additives. Diet contains selenium

[2]Bacitracin methylene disalicylate, a registered product of Zoetics, Parsippany, NJ, USA

Strict biosecurity and biosafety measures were observed during the experiment following the guidelines of the study site. Routine cleaning was done to ensure good sanitation throughout the experiment. Feed and water were given *ad libitum*. Disposal of the birds was done according to the study site procedures.

This trial was a part of a larger study looking at the microbiome, and the traits being studied and reported on in this thesis are: bodyweight of birds at day 14 (initial weight), weekly

bodyweight/bird/cage (day 14, 21, 28, 35 and 42), feed intake/cage (total), bodyweight gain (weekly), mortality and animal health (daily check). Chicken feces were collected weekly per cage into sterile screw tubes and stored at -80^{o}C for further analysis. The final bodyweights of the chickens were taken on day 42. The chickens were euthanized and cecal samples were taken and stored for microbiome profiling.

Data collection was done using forms designed for the research. Growth performance efficiency (ADG, average daily feed intake, gain efficiency, etc.) was calculated and evaluated for each study phase and overall.

3.4 Statistical Analysis

All statistical analyses were done using SAS version 9.4 (SAS Institute Inc., Cary, North Carolina, USA.). Pen means were calculated for all appropriate traits and data on pen means for all traits were subjected to statistical analyses, using models appropriate for RCBD. Block was included in the model as a random effect. The GLM procedure of SAS was used for the analysis of each trait and means for the fixed effects were separated using Tukey's test at a 5% probability level. The key variables used were initial bodyweight (IW), Total Feed Intake (TFI), Bodyweight gain (BWG), and feed conversion ratio (FCR). The following formulas were used to calculate the means for the variables used:

$$\text{IW} = \frac{Pen\ weight\ on\ D14}{Number\ of\ birds\ at\ D14} \quad (1)$$

Where IW is the initial bodyweight of the birds at the start of the treatment phase (day 14).

$$\text{FW} = \frac{Pen\ weight\ on\ D42}{Number\ of\ birds\ at\ D42} \quad (2)$$

Where FW is the final bodyweight of the birds at the end of the experiment (day 42).

TWG = $eqn.(2) - eqn.(1)$ (3)

Where TWG is the total weight gain of the birds.

TFI = $\sum Feed\ issued - feed\ left\ in\ feeder\ at\ day\ 42$ (4)

Where TFI is the total feed intake of the birds.

FCR = $\frac{TFI}{TWG}$ (5)

Where FCR is the feed conversion ratio of the birds.

BWG wk1 = $BW\ at\ D21 - BW\ at\ D14$ (6)

Where BWG wk1 is the bodyweight gain for the first week.

BWG wk2 = $BW\ at\ D28 - BW\ at\ D21$ (7)

Where BWG wk2 is the bodyweight gain for the second week.

BWG wk3 = $BW\ at\ D35 - BW\ at\ D28$ (8)

Where BWG wk3 is the bodyweight gain for the third week.

BWG wk4 = $BW\ at\ D42 - BW\ at\ D35$ (9)

Where BWG wk4 is the bodyweight gain for the final week.

The statistical model used are indicated below:

$Y_{ij} = \mu + B_i + T_j + e_{ij}$ (10)

Y_{ij} = the IW, FW, TWG, TFI, or FCR of the observation (pen mean) from the ith block and the jth treatment.

μ = the overall mean IW, FW, TWG, TFI or FCR

B_i = the random classification effect of the i^{th} block

T_j = the fixed effect of the j^{th} treatment (T1, T2, T3, T4, T5, T6, T7, T8)

e_{ij} = the random residual term, eij ~ $(0, \sigma^2)$

The analyzed data were presented as means, standard error of the mean (SEM), probability values of treatments, and the probability values of blocks. All results from the data were considered to be different significantly at $P < 0.05$.

CHAPTER 4. RESULTS

4.1 Health of experimental animals

After a week of the treatment phase, one mortality was recorded in treatment five (T5R3). The bird did not show any outward clinical signs of sickness. No post-mortem was carried out to identify the actual cause of the death. Apart from that, the rest of the experimental animals were healthy throughout the experiment.

4.2 Growth performance

With regards to the quantity of lysozyme per bird in the group with dietary lysozyme supplementation, each bird consumed 4.620 mg/day of lysozyme in LYZ25A_RHISO, 4.691 mg in LYZ25A_NEOFI, 4.549 mg in LYZ25A_TALPR, 4.368 mg in LYZ25A_PENCH, 4.435 mg in LYZ25A_MYCHI, and 4.624 mg in LYZ25A_POOCH. The birds in the antibiotic supplemented group consumed 8.304 mg/bird/day of BMD.

With regards to the weight and weight gains, there were no significant differences ($P > 0.05$) between the initial, final, and total weight gains of the broiler chickens (Table 5a). However, treatment eight (T8) had a weight gain which was 0.7% and 0.1% higher relative to the control treatment (T1) and T3 respectively.

Results from the feeding trial indicated no significant ($P > 0.05$) differences in the feed intake by the chickens. Treatment 3 (T3) tended to have a higher (> 0.2%) feed consumption relative to the rest of the treatment and treatment five (T5) had the lowest feed consumption relatively.

From the results (Table 5a), there was no significant ($P > 0.05$) difference in the feed to gain ratios (FCR) though, treatment eight (T8) recorded > 0.2% lower FCR relative to the other treatments.

Table 5a: Growth response of broilers on dietary lysozyme supplementation.

Parameter	T1	T2	T3	T4	T5	T6	T7	T8	SEM	P-value[1]	P-value[2]
Initial weight. (g)	420.0	423.4	437.9	432.9	434.7	445.9	418.7	442.9	8.22	0.083	0.158
Final weight. (g)	2872.0	2945.3	3006.8	2930.0	2902.9	2934.5	2984.8	3034.9	71.85	0.487	0.777
Total wt. gain (g)	2452.0	2521.8	2568.9	2497.1	2371.0	2488.7	2566.1	2591.9	66.87	0.410	0.760
Total FI (g)	4135.2	4312.0	4378.6	4246.1	4076.4	4139.1	4315.9	4227.5	95.48	0.425	0.310
FCR	1.689	1.718	1.711	1.712	1.657	1.664	1.683	1.635	0.04	0.108	0.668

Means in a row that do not share a letter are significantly different (P < 0.05); Means in a row without letters are not significantly different (P >0.05)
T1 = Control, T2 = LYZ25A_RHISO**,** T3 = LYZ25A_NEOFI**,** T4 = LYZ25A_TALPR**,** T5 = LYZ25A_PENCH**,** T6 = LYZ25A_MYCHI**,** T7 = LYZ25A_POCCH, T8 = Bacitracin methylene disalicylate BMD (antibiotic)
SEM = Standard error of the means, P-value[1]= P-value (Block), P-value[2] = P-value (Treatment). FI = Feed intake, FCR = Feed conversion ratio

Table 5b: Weekly growth response (bodyweight and weight gain) of broilers on dietary treatment.

Parameter	T1	T2	T3	T4	T5	T6	T7	T8	SEM	P-value[1]	P-value[2]
BW D14	420.0	423.4	437.9	432.9	434.7	445.9	418.7	442.9	8.22	0.083	0.158
BW D21	862.9	879.9	910.0	849.8	857.8	873.9	856.5	911.2	19.10	0.664	0.164
BW D28	1451.1	1497.5	1554.7	1474.2	1470.6	1481.7	1480.0	1562.4	32.25	0.733	0.160
BW D35	2107.1	2164.5	2252.7	2162.5	2115.4	2156.1	2171.8	2289.4	51.11	0.753	0.175
BW D42	2872.0	2945.3	3006.8	2930.0	2902.9	2934.5	2984.8	3034.9	71.85	0.487	0.777
BWG wk1	442.9[ab]	456.5 [ab]	472.2[a]	416.9[b]	423.1 [ab]	428.0 [ab]	437.8 [ab]	468.3 [ab]	12.66	0.832	0.017
BWG wk2	588.2	617.6	644.7	624.5	612.8	607.8	623.5	651.3	16.71	0.297	0.205
BWG wk3	656.0	667.1	698.0	688.3	644.8	674.5	691.9	727.0	24.40	0.348	0.350
BWG wk4	764.9	780.8	754.2	767.5	787.5	778.4	813.0	745.5	32.64	0.379	0.889

Means in a row that do not share a letter are significantly different (P < 0.05); Means in a row without letters are not significantly different (P >0.05)
T1 = Control, T2 = LYZ25A_RHISO**,** T3 = LYZ25A_NEOFI**,** T4 = LYZ25A_TALPR**,** T5 = LYZ25A_PENCH**,** T6 = LYZ25A_MYCHI**,** T7 = LYZ25A_POCCH, T8 = BMD (antibiotic).
SEM = Standard error of the means, P-value[1]= P-value (Block)**,** P-value[2] = P-value (Treatment), BW = Bodyweight, BWG = Bodyweight gain

The breakdown of the bodyweight and weight gain of the broilers per week is indicated (Table 5b). Overall, there were no significant differences ($P > 0.05$) between the weekly bodyweight of the broilers for the various treatments. However, the findings indicate a significant difference ($P = 0.017$) in the bodyweight gain in the first week of the treatment administration.

CHAPTER 5. DISCUSSION

Bodyweight gain and feed conversion efficiency are the most essential phenotypic characteristics observed amongst any antimicrobial feed additives trials (Oliver and Wells 2015). The non-significant difference observed amongst the growth performance parameters observed in (Table 5a) could be attributed to the cecal microbiota contributing to a very small percentage (3 - 7%) of the chickens' gross energy requirement (Xia et al. 2019). Although the results indicated no significant differences in the bodyweight and weight gains of the broilers, birds in the antibiotic supplemented group (T8) followed by LYZ25A_NEOFI (T3) recorded a relatively higher weight gains amongst the other treatments including the controls (Table 5a and 5b). This observation may be due to the high antimicrobial and growth-promoting effects of the treatments resulting from increased antioxidant and immune genes of the gut as well as increased intestinal villi which enhance absorption of nutrients (Pan and Yu 2014). This is in line with the findings of (Xia et al. 2019) as there was no significant difference ($P > 0.05$) in the bodyweight gain when broiler chickens were fed with dietary lysozymes or antibiotics but their performance were better numerically when compared to the control. This non-significant ($P > 0.05$) difference reported in the result is in contrast to the findings of Abdel-Latif et al. (2017), as diets supplemented with exogenous enzymes (lysozyme) were fed to broiler chickens and the animals fed with the control diets showed a reduced bodyweight gain. In addition, other findings indicated that chickens supplemented with growth enhancers had an improved bodyweight ($P < 0.05$) than those in the control group (Khan et al. 2011; Shahir et al. 2014; Amerah et al. 2012; Salim et al. 2013).

The significant difference ($P = 0.017$) observed in bodyweight gain (Table 5b) in the first week (BWG wk1) indicated that T3 (LYZ25A_NEOFI) had a relatively higher (> 0.1%) average BWG than other treatments especially T4 (LYZ25A_TALPR) which was about 1.6% lower than

T3. This difference may be due to the composition of the enzyme (LYZ25A_NEOFI) and the target substrate in the feed since enzymes work on a specific substrate. In addition, young animals tend to have a weaker digestive system which does not allow efficient digestion and assimilation of nutrients. For example, young animals (birds) can not efficiently digest diets containing high levels of fibers. As they mature (age), their system can tolerate high fiber levels even when there are no fibrinolytic enzymes added to their diets.

Feed intake is directly influenced by the energy content of the diets fed to animals. The non-significant effect observed in the feed intake of the broilers might be attributed to the same energy content of the diets since they were formulated to be isocaloric and isonitrogenous. Table (3) indicated the energy content of the diets. This is in line with the findings from Abdel-Latif et al. (2017) when exogenous dietary lysozymes were fed to broiler chickens and there was no significant difference in the feed intake compared to the control group. In addition, when antibiotics or lysozymes were added to the diets of broilers, there were no significant ($P > 0.05$) effects on their feed intake (Gong et al. 2017). Also, feed intake was unaffected ($P > 0.05$) when broiler chickens were fed with dietary lysozymes or antibiotics (Xia et al. 2019). This result is in contrast to the findings of Altaf et al. (2019) as feed intake was significantly ($P < 0.05$) lower in birds fed with diets supplemented with growth enhancers like synbiotics. The difference was attributed to the improvement of the biological functions of the essential microbes in the gut of the host chicken and enhancement of nutrient absorption and hence, reducing the feed intake significantly (Altaf et al. 2019). A significant difference ($P < 0.05$) in feed intake was also reported when growth promoters were supplemented to broiler chickens as broilers in the “growth promoters” supplemented group had a decreased feed intake (Park and Kim 2014).

From the results, the dietary supplementation with lysozyme or antibiotics had no significant ($P > 0.05$) effects on the FCR. This result is in line with the findings from Xia et al. (2019) as a non-significant difference in FCR was recorded when broiler chickens were fed with dietary lysozymes or antibiotics. A similar result ($P > 0.05$) was achieved when antibiotic or lysozyme was added to the diets of broilers (Gong et al. 2017). In contrast to this, there was a significant ($P < 0.05$) improvement of the FCR when broiler chickens were given diets supplemented with dietary lysozyme (Abdel-Latif et al. 2017).

The non-significant differences observed among the various parameters may also be attributed to the adherence to strict biosecurity and biosafety measures observed throughout the experiment. This confirms that the risk of disease-causing pathogens in broiler production is significantly reduced by good biosecurity techniques (Bojesen et al. 2003; Graham et al. 2008; Newell et al. 2011). The high level of biosecurity means that the birds were not challenged enough so the benefit of antibiotics and/or the lysozymes may not have been fully realized that's why they were similar to the un-supplemented treatments. In addition, when broilers are exposed to a stress-free environment, the administration of in-fed antibiotics or other alternatives may not increase their growth performance (Gong et al. 2017).

The results which indicated non-significant differences amongst the various treatments implies that the performance of the test products (lysozymes) is as good as the two controls (T1 and T8) used in the experiment and could be used as an alternative product for growth promotion in broilers.

CHAPTER 6. CONCLUSION

The aim of the research was to evaluate the use of fungal lysozyme as a potential replacement for in-feed antibiotics used as growth promoters in the chicken industry. It is concluded from this research findings that the use of lysozyme as an alternative to antibiotics statistically ($P > 0.05$) showed no superior improvement on all the parameters measured. The performances were similar among the eight treatments and hence, antibiotics and lysozyme did not improve broiler performance.

Notwithstanding, the lysozyme supplemented groups (especially LYZ25A_NEOFI) had better performance than the control groups numerically suggesting that there may be an appreciable improvement when optimum supplementation or inclusion is given. Due to the health, social and economic implications of the use of antibiotics in animal production to producers and consumers, the use of lysozymes (especially LYZ25A_NEOFI) as growth promoters (which has no proven residual effects and are from a natural origin) in broiler production would be helpful to ensure cost-effective animal production, safe and quality food for consumers. A feeding trial testing for different dose rates of the LYZ25A_NEOFI lysozyme as a growth promoter in broiler diets would be recommended in future studies.

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APPENDICES

GROWTH PARAMETERS

APPENDIX 1: Data for total feed intake.

TOTAL FEED INTAKE (g)								
	T1	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**
Cage 1	7502	7331	9063	8856	8282	7997	7912	8845
Cage 2	7709	8708	8384	7866	9040	8377	8608	9152
Cage 3	8955	8864	8721	8465	5575	8165	8401	8584
Cage 4	8557	9051	8115	8715	8437	8664	8747	8981
Cage 5	7673	7825	9164	9094	7925	7503	9187	7781
Cage 6	9228	9055	9099	8590	8947	8634	9018	8530
Cage 7	7389	8993	8801	8749	7713	7912	9158	8474
Cage 8	8645	8632	8468	8698	8628	9127	7314	7654
Cage 9	8501	8904	8798	8235	8415	8575	9225	8564
Cage 10	8544	8876	8958	7653	8566	7827	8748	7984

APPENDIX 2: Data for bodyweight at D14.

Bodyweight D14								
	T1	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**
Cage 1	405	380	460	441	428	516	343	503
	456	428	445	477	448	448	441	444
Cage 2	393	398	445	389	463	395	452	490
	398	502	370	409	482	410	386	453
Cage 3	473	407	487	472	402	464	380	440
	426	477	448	463	455	508	465	422
Cage 4	426	445	416	498	383	493	407	461
	439	388	454	386	450	437	433	450
Cage 5	368	362	467	428	446	362	395	510
	464	384	425	462	495	473	478	427
Cage 6	451	407	460	395	470	466	414	434
	373	406	423	471	433	394	436	390
Cage 7	364	428	397	436	365	380	443	451
	347	440	421	434	423	381	415	368
Cage 8	436	511	412	450	454	484	421	386
	456	434	435	429	450	493	380	439
Cage 9	466	430	517	448	375	413	461	521
	456	390	356	413	416	504	419	412
Cage 10	363	439	495	367	422	388	431	432
	440	412	424	390	434	508	374	425

APPENDIX 3: Data for bodyweight at D21.

Bodyweight D21								
	T1	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**
Cage 1	814	792	942	843	851	1002	870	916
	790	870	960	925	928	874	727	999
Cage 2	879	879	884	824	930	786	812	1039
	809	962	747	810	968	823	957	1019
Cage 3	974	867	945	931	766	910	772	876
	945	1010	981	897		942	944	894
Cage 4	859	805	869	1044	777	970	845	918
	896	802	963	758	944	866	824	959
Cage 5	741	738	919	837	878	685	822	930
	860	772	950	921	959	855	953	869
Cage 6	985	825	985	778	926	905	876	892
	783	873	838	715	881	820	905	794
Cage 7	713	918	880	902	757	793	953	941
	757	950	870	839	793	737	845	790
Cage 8	875	1049	860	905	750	930	884	786
	920	927	912	870	975	950	770	910
Cage 9	920	913	1075	830	739	845	906	1080
	986	832	740	809	865	960	821	859
Cage 10	868	920	981	733	886	817	840	886
	884	894	899	824	816	1007	803	866

APPENDIX 4: Data for bodyweight at D28.

Bodyweight D28								
	T1	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**
Cage 1	1402	1432	1596	1452	1454	1590	1510	1662
	1296	1368	1552	1690	1589	1493	1306	1709
Cage 2	1481	1549	1583	1504	1691	1343	1500	1745
	1324	1680	1258	1317	1593	1501	1552	1775
Cage 3	1544	1465	1560	1549	1344	1506	1339	1535
	1501	1703	1669	1610		1578	1549	1633
Cage 4	1482	1313	1452	1777	1410	1694	1517	1618
	1511	1420	1741	1280	1637	1543	1503	1630
Cage 5	1290	1242	1599	1525	1506	1092	1369	1571
	1502	1347	1504	1580	1568	1517	1623	1451
Cage 6	1808	1410	1604	1349	1630	1542	1458	1585
	1381	1572	1460	1267	1531	1488	1633	1384
Cage 7	1180	1676	1602	1492	1262	1362	1627	1552
	1313	1625	1486	1539	1304	1269	1521	1376
Cage 8	1432	1442	1520	1558	1258	1579	1417	1285
	1614	1554	1535	1521	1696	1538	1241	1488
Cage 9	1524	1570	1842	1401	1262	1497	1662	1832
	1528	1427	1297	1428	1480	1484	1387	1452
Cage 10	1486	1507	1637	1286	1475	1388	1490	1516
	1423	1647	1596	1359	1377	1629	1395	1449

APPENDIX 5: Data for bodyweight at D35.

Bodyweight D35								
	T1	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**
Cage 1	1969	1940	2295	2090	2115	2226	2226	2437
	1772	1776	2249	2602	2223	2022	1938	2387
Cage 2	1827	2258	2336	2010	2489	1957	2243	2475
	1960	2420	1810	1967	2246	2343	2170	2665
Cage 3	2281	2112	2168	2226	1989	2064	1976	2233
	2245	2427	2442	2353		2356	2092	2480
Cage 4	2179	1974	2004	2467	2005	2428	2189	2432
	2222	2037	2419	1947	2395	2127	2221	2405
Cage 5	1838	1604	2270	2312	2051	1651	2095	2190
	2257	1898	1999	2240	2132	2230	2354	2210
Cage 6	2649	2088	2238	1940	2436	2124	2126	2403
	2058	2374	2222	1926	2054	2262	2455	2141
Cage 7	1736	2511	2390	2104	1823	2133	2356	2193
	1971	2330	2246	2314	1796	1840	2243	2169
Cage 8	1887	2066	2142	2355	1863	2368	1999	1868
	2393	2343	2330	2333	2478	2346	1772	2190
Cage 9	2401	2404	2748	1988	2037	2218	2512	2503
	2164	2103	1906	2164	1990	2188	2074	2151
Cage 10	2220	2223	2408	1936	2193	1956	2256	2162
	2113	2402	2431	1975	2003	2283	2139	2094

APPENDIX 6: Data for bodyweight at D42.

Bodyweight D42								
	T1	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**
Cage 1	2782	2707	3128	2893	2872	2884	2947	3308
	2480	2442	3064	3468	3173	2811	2741	3109
Cage 2	2700	3053	3215	2606	3360	2603	2990	3254
	2753	3186	2428	2620	3109	3268	2881	3693
Cage 3	3185	2828	2894	3096	2863	2688	2794	2752
	3142	3306	3288	3122		3223	3325	2892
Cage 4	3058	2832	2637	3123	2987	3350	3177	3342
	3000	2735	2958	2809	3211	2944	3121	3156
Cage 5	2439	2218	2888	3091	2749	2315	2866	2931
	2922	2567	2495	3142	2761	2924	3148	2683
Cage 6	3431	2814	3046	2435	3347	2971	3107	3020
	2700	3291	2928	2501	2777	3141	3210	2819
Cage 7	2234	3314	3197	3175	2539	3100	3179	3059
	2641	3016	3079	3123	2349	2452	3095	3029
Cage 8	2497	2712	2959	3278	2487	3237	2587	2559
	3250	3303	3261	3161	3262	3220	2457	2981
Cage 9	3351	3308	3573	2640	2702	3087	3160	3300
	2928	2961	2654	2968	2823	2943	2879	3022
Cage 10	3115	3064	3137	2667	3128	2617	3096	2886
	2832	3248	3307	2681	2695	2912	2936	2902

APPENDIX 7: ANOVA table for initial weight.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
CAGE	9	11044.450	1227.161	1.81	0.0831
TREATMENT	7	7477.938	1068.227	1.58	0.1581
Error	63	42616.500	676.452		
Total	79	61138.888			

APPENDIX 8: ANOVA table for final weight.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
CAGE	9	442384.375	49153.819	0.95	0.4877
TREATMENT	7	205936.150	29419.450	0.57	0.7777
Error	63	3252726.225	51630.575		
Total	79	3901046.750			

APPENDIX 9: ANOVA table for total weight gain.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
CAGE	9	42354.388	47060.043	1.05	0.4098
TREATMENT	7	185199.738	26457.105	0.59	0.7602
Error	63	281680.762	44712.076		
Total	79	3425600.888			

APPENDIX 10: ANOVA table for total feed intake.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
CAGE	9	847008.313	94112.035	1.03	0.4248
TREATMENT	7	772759.238	110394.177	1.21	0.3101
Error	63	5743464.138	91166.097		
Total	79	7363231.688			

APPENDIX 11: ANOVA table for feed conversion ratio.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
CAGE	9	0.193	0.021	1.70	0.1078
TREATMENT	7	0.062	0.009	0.70	0.6682
Error	63	0.797	0.013		
Total	79	1.053			

APPENDIX 12: ANOVA table for bodyweight D21.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
CAGE	9	24571.953	2730.217	0.75	0.6635
TREATMENT	7	39824.097	5689.157	1.56	0.1642
Error	63	229857.372	3648.530		
Total	79	294253.422			

APPENDIX 13: ANOVA table for bodyweight D28.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
CAGE	9	62680.591	6964.510	0.67	0.7333
TREATMENT	7	114514.122	16359.160	1.57	0.1602
Error	63	655546.534	10405.501		
Total	79	832741.247			

APPENDIX 14: ANOVA table for bodyweight D35.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
CAGE	9	151934.566	16881.618	0.65	0.7533
TREATMENT	7	278738.147	39819.735	1.52	0.1754
Error	63	1645782.509	26123.532		
Total	79	2076455.222			

APPENDIX 15: ANOVA table for bodyweight gain wk1.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
CAGE	9	7931.466	881.274	0.55	0.8323
TREATMENT	7	30036.697	4290.957	2.68	0.0172
Error	63	100984.959	1602.936		
Total	79	138953.122			

APPENDIX 16: ANOVA table for bodyweight gain wk2.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
CAGE	9	30767.263	3418.585	1.22	0.2970
TREATMENT	7	28211.550	4030.221	1.44	0.2045
Error	63	176011.138	2793.828		
Total	79	234989.950			

APPENDIX 17: ANOVA table for bodyweight gain wk3.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
CAGE	9	61153.675	6794.853	1.14	0.3478
TREATMENT	7	47481.850	6783.121	1.14	0.3503
Error	63	37492.025	5951.937		
Total	79	483607.550			

APPENDIX 18: ANOVA table for bodyweight gain wk4.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
CAGE	9	104976.941	11664.105	1.10	0.3793
TREATMENT	7	31023.422	4431.917	0.42	0.8888
Error	63	671004.734	10650.869		
Total	79	807005.097			