Effects of selected zootechnical feed additives as alternatives to zinc-bacitracin antibiotic growth promoter in broiler diets

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Thesis accepted in fulfilment of the requirements for the degree Masters of Science in Animal Science at the North-West University

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DECLARATION

I hereby declare that the work contained in this research dissertation is my own original work for a degree of Masters of Science in Agriculture in Animal Science working under the supervision of Professor Victor Mlambo. This dissertation has not been previously submitted to any University. Materials and information from any other sources have been fully recognised.

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GENERAL ABSTRACT

This study was designed to investigate the effects of selected zootechnical feed additives [probiotic (live Bacillus subtilis), organic acids, protease enzyme and chelated minerals] combinations as alternatives to zinc-bacitracin (ZnB) antibiotic growth promoter in broiler diets on growth performance, blood parameters, meat quality and tibia bone parameters. Eight hundred Cobb broiler chicks were evenly distributed to 40 pens to which five dietary treatments: negative control (T1) (commercial broiler diets with no antibiotics); T2 (positive control (commercial broiler diets with zinc-bacitracin); T3 (T1 + chelated minerals + protease enzyme); T4 (T1 + chelated minerals + protease + organic acids) and T5 (T1 + chelated minerals + protease + probiotic) were randomly allocated. The inclusion levels of chelated minerals, protease enzyme, organic acids and probiotic were 0.03, 0.05, 0.5 and 0.02%, respectively. The chelated minerals additive was a composite of 19.3% copper, 36% zinc, and 44.7% manganese by weight. A maize grain-soybean meal-based starter diet was fed to chicks from 0-13 days of age. On days 14, 15 and 16, after a 16 hour fast, the birds were challenged with a high protein diet (40% soybean meal and 10% poultry by-products) and a finisher diet was fed thereafter till day 35. Intake and weight gain data were used to calculate average daily feed intake (ADFI), feed conversion ratio (FCR) and average daily gain. There was no week × diet interaction effect on feed intake (FI), body weight gain (BWG) and FCR. There were no dietary effects ($P>0.05$) on FI, average daily gain and FCR. Haematological parameters influenced ($P<0.05$) by the diet were haemoglobin and haematocrit (HTC) only. Chickens fed T5 (9.57 ± 0.32 g/dl) and T1 (9.52 ± 0.32 g/dl) had the highest haemoglobin levels while T2 resulted in the lowest levels. T1 (0.37 ± 0.01%) also caused the highest number of haematocrit followed by the T5 (0.36 ± 0.01%) and T2 (0.31 ± 0.1%) resulted the lowest value of haematocrit. Dietary treatments did not have any effect ($P>0.05$) on erythrocytes, leucocytes,

neutrophils, lymphocytes, monocytes, eosinophils, basophils and normoplasts. The serum biochemistry indices, alanine transaminase (ALT), sodium and total serum protein were significantly ($P<0.05$) influenced by the dietary treatments but not aspartate aminotransferase (AST), potassium, albumin, urea, calcium and cholesterol. T2 (8.50 ± 1.57 IU/L) resulted in the highest level of ALT compared to the lowest of treatment T3 (2.25 ± 1.57 IU/L). Feeding T1 (33.37 ± 1.14 g/l) caused the highest total protein meanwhile T2 (29.75 ± 1.14 g/l) diet resulted in the least total protein. The T1 continues with same trend of causing the highest level even in sodium while T3 (144.00 ± 1.25 mmol/l) resulted in the lowest value. The T1 (0.39 ± 0.05 mmol/l) diet also had the highest urea levels and T3 (0.20 ± 0.05 mmol/l) the lowest. There were no dietary effects ($P>0.05$) on bone development parameters. Similarly, dietary treatments did not influence ($P>0.05$) all carcass traits. Diet had no effect ($P>0.05$) on all internal organs apart from the spleen and proventriculus. The highest weight of spleen was observed in chickens fed T3 (3.81 ± 0.32 g) while T1 (2.12 ± 0.32 g) had the lowest weight. T3 (12.63 ± 0.61 g) had the heaviest proventriculus weight while T2 (9.63 ± 0.61) had the lowest weight. The results of external organs of broilers also showed a lack of significant effect ($P>0.05$) of diet on breast, drumstick, wing, head and shank weights. However, diet had an effect ($P<0.05$) on neck weight; T1 (55.50 ± 2.08 g) promoted the heaviest necks while T3 (44.88 ± 2.08 g) promoted the lightest neck weight. With regards to meat quality measurements, there was no dietary effect ($P >0.05$) on pH, dripping loss and shear force. All meat colour parameters were not influenced by diet apart from redness. Overall, the proposed zootechnical feed additives were shown to have potential as alternatives to zinc-bacitracin antibiotic growth promoter in broiler diets. Collectively, the results of this study can be used in informing formulating antibiotic-free diets that will not have any negative effects on growth performance and the meat quality of broilers.

Keywords: Broilers, antibiotic growth promoters, zootechnical feed additive, growth, blood, meat.

ACKNOWLEDGEMENTS

I would like to thank Prof Victor Mlambo, my major supervisor, for his continuous guidance and wisdom throughout the course of my studies, for without his help this would not have been possible. His assistance during my studies, research projects and preparation of my dissertation were vital and I thank him. I would also like to thank my co-supervisor Ms Natasha Snyman for her dedication in helping to make this study a success. Let me also express my gratitude to the following institutions for their involvement in the completion of this study: The National Research Foundation (Scarce Skills Scholarship), Novus International and NWU-Post Graduate Bursary.

I would also like to appreciate my fellow colleagues: Daniel Matlou, Amogelang Ratanang Disetlhe, and Isaac Thapelo Matona, for dedicating their valued time to ensure that the trial and the analysis of this study was a success. I also wish to thank Prof. U. Marume (Animal Science programme) for assisting with meat colour apparatus and Dr M. Nyirenda (Animal Health programme) for his assistance during the bone structure analysis (data collection and instrument operations).

I am really grateful to my fellow postgraduate students who willingly helped during the experimental work and shared valued advice: Mr T.T. Lebeya, Mr L. Papalagae, Ms Q. Dlamini, Ms B.B. Masilo, Mr T.B. Matshogo and Ms M. Makhafola, the broiler unit supervisor.

I thank God Almighty for granting me the opportunity and strength to complete this study. I would also like to thank my parents Mr L.J. and Mrs P.M. Thema and my three siblings Mollale, Moditja and Moyahabo Thema for belief, support and patience during the period of my studies. You are very special people and may the good Lord shower you with more blessings.

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LIST OF ABBREVIATIONS

AGP	Antibiotic growth promoters
BWG	Body weight gain
EU	European Union
FCR	Feed conversion ratio
FI	Feed intake
GI tract	Gastro-intestinal tract
NC	Negative control (without antibiotic growth promoters)
NRC	National Research Council
NSP	Non-starch polysaccharides
PCZnB	Positive control with zinc-bacitracin
WHC	Water holding capacity
ZnB	Zinc-bacitracin antibiotic

1 CHAPTER 1 - GENERAL INTRODUCTION

1.1 Background

The main goal in livestock production is to maximise yields at a low cost without compromising quality of the product. The use of drugs and similar products, as feed additives, to enhance growth are important strategies for achieving this goal (Huyghebaert et al., 2011), however the integrity in application of drugs in livestock production is in question. In broiler production, the use of feed additives results in increased body weight gain (BWG) over a short period of time, often accompanied by lower feed intake (FI). Broilers are susceptible to micro-organisms such as Salmonella spp., Escherichia coli and Clostridium perfringens, which establish a pathogenic microbiota population in the small intestine resulting in poor digestion and competition with the host for nutrients (Engberg et al., 2000). Traditionally, antibiotics have been used to control these pathogenic micro-organisms due to their antimicrobial effect and are also used as growth promoters.

Antibiotics can be defined as naturally-occurring semi-synthetic and synthetic compounds with antimicrobial activity that can be administered orally, parentally or topically. They are used in human and veterinary medicine to treat or to prevent diseases, and for other purposes including growth promotion in animal feed (Phillips et al., 2003). Antibiotic growth promoters (AGP) have been widely used in poultry feed for the last 50 years (Yegani & Korver, 2008). Antibiotics are used therapeutically to improve the general health and well-being of animals, but are mostly given for prophylactic or methaphylaxis purposes to avoid risk of infections. They are also given to improve growth rate and feed conversion efficacy (as antimicrobial growth performance promoters) (Huyghebaert et al., 2011).

1.2 Problem statement

The use of antibiotics as growth promoters in animal feed has raised concerns about increased resistance by pathogens to antibiotics and the existence of antibiotic residues in animal products (Truscott & Al-Sheikhly, 1977; Waldroup et al., 1985; Powell et al., 1998; Hernández et al., 2005; Pirgozliev et al., 2008). These concerns saw the European Union (EU) ban AGPs in 2006 (Castanon, 2007), which resulted in animal performance problems caused by poor feed conversion efficiency and a rise in animal diseases such as (subclinical) necrotic enteritis (Wierup, 2001; Dibner & Richards, 2005). Therefore, viable alternatives to AGPs must be identified and introduced to enhance animal performance. Chelated trace minerals, organic acids, prebiotics, probiotics, herbs and etheric oils may have the potential to increase performance, improve resistance to pathogenic bacterial colonisation and enhance mucosal immunity, consequently reducing pathogenic load and improving the health status of animals (Yalcinkaya et al., 2008; Huyghebaert et al., 2011).

1.3 Justification

Various studies (Huyghebaert et al., 2011; Demir et al., 2003) have been conducted to determine the effects of alternatives to AGPs on growth indices and their general effect on the microbiota and carcass characteristics of broilers (Ashayerizadeh et al., 2011). However, there is a paucity of information on the effectiveness of combinations of organic acids, enzymes and probiotic feed additives as alternatives to AGPs in broiler chickens. Such combinations may result in positive associative effects, thus enhancing the effectiveness of these feed additives as substitutes for AGPs. Consumers are concerned about the role of antibiotic feed additives in the proliferation of

drug-resistant pathogenic bacteria. This has resulted in a high demand for antibiotic-free animal products.

1.4 Research aim

The aim of the current study is to investigate the effects of an organic acid, a probiotic, minerals, and enzymes and their combinations on the performance, organ size, bone development and meat characteristics in broilers, as alternatives to AGPs. The effect of these feed additives will be compared to Zn-bacitracin, a commonly used AGP in broiler diets.

1.4.1 Specific research objectives

To evaluate the effects of combinations of feed additives [organic acid, a probiotic (Bacillus subtilis), chelated minerals (Zn, Mn and Cu) and protease enzyme] as alternatives to AGPs in broiler diets on:

1. Growth performance, feed intake (FI) and feed utilisation efficiency
2. Blood biochemistry and general blood count
3. Carcass traits and bone parameters.

1.5 Research hypothesis

The proposed zootechnical feed additives will improve the production parameters, feed utilisation efficiency, bone parameters and meat quality in broiler chickens.

1.6 References

Ashayerizadeh, A., Dabiri, N., Mirzadeh, K.H. & Ghorbani, M.R., 2011. Effect of dietary supplementation of probiotic and prebiotic on growth indices and serum biochemical parameters of broiler chickens. J. Cell. Anim. Biol. 5(8), 152-156.

Castanon, J.I**.,** 2007. History of the use of antibiotic as growth promoters in European poultry feeds. Poultry Sci. 86(11), 2466-2471.

Dibner, J.J. & Richards, J.D., 2005. Antibiotic growth promoters in agriculture: history and mode of action. Poultry Sci. 84, 634-643.

Engberg, R.M., Hedemann, M.S., Leser, T.D. & Jensen, B.B., 2000. Effect of zinc bacitracin and salinomycin on intestinal microflora and performance of broilers. Poultry Sci. 79, 1311-1319.

Hernandez, G., Altmann, M., Sierra, J.M., Urlaub, H., Diez Del Corral, R., Schwartz, P. & Rivera-Pomar, R. 2005. Functional analysis of seven genes encoding eight translation initiation factor 4E (eIF4E) isoforms in Drosophila. Mech. Dev. 122(4), 529-543.

Huyghebaert, G., Ducatelle, R. & Van Immerseel, F., 2011. An update on alternatives to antimicrobial growth promoters for broilers. Vet. J. 187(2), 182-188.

Phillips, I., Casewell, M., Cox, T., De Groot, B., Friis, C., Jones, R., Nightingale, C., Preston, R. & Waddell, J., 2004. Does the use of antibiotics in food animals pose a risk to human health? A critical review of published data. J. Antimicrob. Chemoth, 53(1), 28-52.

Pirgozliev, V., Oduguwa, O., Acamovic, T. & Bedford, MR., 2008. Effects of dietary phytase on performance and nutrient metabolism in chickens. Brit. Poultry Sci. 49, 144-154.

Powell, J., Miles, R. & Siriwardena, A., 1998. Antibiotic prophylaxis in the initial management of severe acute pancreatitis. Brit. J. Surg. 85, 582-587.

Truscott, R.B. & Al-Sheikhly, F., 1977. Reproduction and treatment of necrotic enteritis in broilers. Am. J. Vet. Res. 38(6), 857-861.

Waldroup, P.W., Ramsey, B.E., Helwig, H.M. & Smith, N.K., 1985. Optimum processing for soybean meal used in broiler diets. Poultry Sci. 64, 2314-2320.

Wierup, M., 2001. The Swedish experience of the 1986-year ban of antimicrobial growth promoters, with special reference to animal health, disease prevention, productivity, and use of antimicrobials. Microb. Drug Resist. 7, 183-190.

YalcInkaya, I., Gungor, T., Balsann, M. & Erdem, E., 2008. Mannan oligosaccharides (MOS) from Saccharomyces cerevisiae in broilers: effects on performance and blood biochemistry. Turk. J. Vet. Anim. Sci. 32, 43-48.

Yegani, M. & Korver, D.R., 2008. Factors affecting intestinal health in poultry. Poultry Sci. 87, 2052-2063.

2 CHAPTER 2 - LITERATURE REVIEW

2.1 Introduction

Around the world, poultry production is amongst the largest growing segments of the animal industry. Advances in genetics, nutrition, housing and marketing of broiler chickens have fuelled the expansion of the poultry industry. This has led to the growth of more controlled and intensive production systems designed to increase number of birds produced within a short time frame using minimum space (Moore et al., 1946). However, increased production has led to outbreaks in a number of diseases such as necrotic enteritis (Bray, 2008). As a result, antibiotic growth promoters (AGPs) were introduced to control disease outbreaks after animals fed dried mycelia of Streptomyces aureofaciens demonstrated substantial improvements in growth (Castanon, 2007). Moore et al. (1946) and Jukes et al. (1950) were the first to report the beneficial effect on production efficiency of feeding antibiotics at sub-therapeutic levels.

2.2 Enteric diseases

Disease is defined as any malfunction or disturbance of the normal structure and function of any part, organ or system within the host (Hoerr, 1998). Enteric diseases refer to the malfunction of the gastrointestinal tract (GI tract) (Hoerr, 1998). In poultry production, enteric diseases have a negative impact on production and also pose a threat to human health through food-borne illnesses (Patterson & Burkholder, 2003). All these contribute to an increase in the costs related to poultry production.

The primary functions of the GI tract are to carry out the processes of digestion and absorption of nutrients, which are both influenced by the transit time of the digesta, pH and changes in the net

absorption of water (Hoerr, 1998). In addition, the GI tract can act as a barrier, protecting internal organs from exposure to pathogens found in the lumen. It also represents the largest mass of lymphoid tissue in the body, known as the gut-associated lymphoid tissue (GALT) (Salminen et al., 1998). Pathogens are forced to content with a number of the host's natural defence mechanisms to cause disease hence GIT also provide immune function. A decreased gastric pH, rapid transit through portions of the GI tract, competitive microbiota and GALT have a synergistic relationship that prevents pathogens from causing disease. Besides these pathogens (bacteria, fungal, viral and parasitic), enteric disease can result from nutritional factors, stress, injury and ingestion of toxins. These factors may not be direct causes of disease, but will predispose the bird to the disease (Wiley et al., 2011). The duration and severity of stress components, such as harsh environmental conditions, inappropriate handling and very high or low temperatures can increase the susceptibility to diseases (Wiley et al., 2011).

2.3 The gastrointestinal microflora

The GI tract of simple non-ruminants consists of a large variety of microbial species. In broilers, microbial species number over 650 species with over half being of unknown genera (Apajalahti et al., 2004). The stomach, initially was thought to be free from indigenous microflora but it is now known that it is in fact resident to a number of microorganisms, albeit in low numbers compared to the colon (Lee et al., 1993). Likewise, the proximal large part of the small intestine was once thought to be free of a resident microflora. The microbes begin to colonize the gastrointestinal tract of the chicken at hatching and the synergy between the host and the microflora progresses throughout lifecycle of the bird.

At hatching, chicks are sterile and the microflora develops slowly thereafter. Dietary components are the main suppliers of energy to the bacteria present in the GI tract. These dietary components are either resistant to the animal's endogenous enzymes or slowly digested and, as a result, the composition and structure of the diet plays a role for the microbial community of this ecosystem (Apajalahti et al., 2004). The effect of diet is easily observed in the microbial community structure and diversity (Gibson et al., 1996; Reid & Hillman, 1999; Apajalahti et al., 2001).

Apajalahti et al. (2001) indicated that within 24 hours of hatching, both cecal and ileal bacteria counts can reach as high as 1010 and 108 per gram of digesta, respectively. During the first three days, these numbers tend to increase, reaching between 109 and 1011. Even though the numbers remain relatively stable, the overall composition of the microflora undergoes a lot transformation over the same time of period (Apajalahti et al., 2001). Lu et al. (2003) showed that the age of the bird could directly have an impact on the microbial community stability based on profiles obtained from 16s gene sequence. Enterococci and lactobacilli have been shown to be high in the crop, and the lower portion of the small intestine, while coliforms, enterococci and lactobacilli are the predominant microorganisms in the cecum (Barnes, 1972; Mead & Adams, 1975; Van der Wielen et al., 2002).

2.4 Antibiotic use in poultry

Antibiotics in poultry production can be used therapeutically or as growth promoters (NRC, 1980). Antibiotics that are fed to promote growth and for prevention of diseases are given at low doses (sub-therapeutic) compared to those given for therapeutic purposes. The in-feed antibiotics have the capacity to suppress certain microbial communities (Hofacre et al., 2006). However, antibiotic

use has the potential to induce bacterial resistance. Commercial poultry producers primarily include AGPs in feed in order to enhance feed efficiency and growth rates (Stutz et al., 1984).

An increase in demand by consumers for chickens raised without antibiotics resulted in the need for non-antibiotic alternatives, such as organic acids, probiotics, prebiotics, natural antibacterials and enzymes to inhibit the growth of pathogenic intestinal bacteria such as E. coli and Clostridium perfringens (Elwinger et al., 1992; Hofacre et al., 1998). These alternatives, in combination with management changes, may improve gut health and promote uniformity in terms of growth performance of chickens (Casewell et al., 2003).

Therapeutic antibiotics are given in case of a disease outbreak on a farm. The veterinarian will determine whether the birds can be treated with an antibiotic and if so, which antibiotic and by using what route of administration (in feed or via drinking water). The type of antibiotic to be used is frequently constrained by the stability between the value of the drug and the severity of the disease incidence. Antibiotic decisions can also be influenced by using the regulated duration of withdrawal before slaughter to make certain that no drug residue is found in the meat.

Recent studies have suggested that the positive effects of AGP have become more profound (Graham et al., 2007). This is due to further improvements in management, genetics and facilities (Graham et al., 2007). A counter argument to the use of AGPs is that the practice increases the risk of endemic bacterial populations developing resistance to the antibiotics, which is one possible hypothesis for the apparent loss of efficacy of these compounds. There is evidence that consistently feeding AGPs for several years could result in the loss of their ability to have a positive effect on growth performance (Marshall & Levy, 2011; Waibel et al., 1954; Libby & Schaible, 1955). This loss of efficacy was shown to be reversible when a different AGP was used, indicating that the

problem might be related to development of antibiotic resistance (Nesse *et al.*,,,,,,,,,, 2015; McGinnis *et al.*,,,,,,,,,, 1958). As early as 1951, indications of emerging antimicrobial resistance in animals began with turkeys fed streptomycin (Starr & Reynolds, 1951). Between 1958 and 1959 similar resistance was observed in chickens fed tetracycline as an AGP (Wong, 2001).

Table 2.1. Therapeutic antibiotics used in poultry production

Antibiotic	Egg layer chickens			Broiler chickens		
	Feed	Drinking water	Growth promotant	Feed	Drinking water	Growth promotant
Bacitracin	Yes	Yes	No	Yes	Yes	Yes
Bambermycin	No	No	No	Yes	Yes	Yes
Chlortetracycline	Yes	No	No	Yes	Yes	Yes
Erythromycin	No	No	No	Yes	Yes	No
Enrofloxacin	No	No	No	No	Yes	Yes
Lincomycin	No	No	No	Yes	Yes	Yes
Neomycin	No	No	No	No	Yes	No
Novobiocin	No	No	No	Yes	No	No
Oxytetrecycline	No	No	No	Yes	Yes	Yes
Penicillin	No	No	No	Yes	No	Yes
Streptomycin	No	No	No	No	Yes	No
Sulfonamides	No	No	No	Yes	Yes	No
Tylosin	Yes	No	Yes	Yes	No	Yes
Virginiamycin	No	No	No	No	No	Yes

(Code of Federal Regulations, 2005)

2.5 Mode of action of Antibiotic growth promoters

Antibiotic growth promoters have been reliably used in poultry production over the years because of their impact on promoting growth and protecting the health of broiler chickens (Rosen, 1996; Engberg et al., 2000; Dibner et al., 2005). The mode of action for AGPs has been described as an interaction between the antibiotics and the intestinal micro-flora (Castanon, 2007). The four proposed mechanisms for their effects on increase overall performance (Niewold, 2007) are as follows:

1. Inhibiting endemic subclinical infection, therefore decreasing the metabolic cost of the innate immune system.
2. Reducing metabolites that are produced by microbes, which supress animal growth, such as bile degrading products and ammonia.
3. Enhancing the absorption and the usage of nutrients, by thinning of the intestinal wall in AGP-fed animals.
4. Reducing nutrient use by pathogenic microbes.

An emerging thought suggests that reduced enteric inflammation is responsible for the benefits associated with the dietary addition of AGP (Nieworld, 2007). The imbalance of the microflora is usually caused by changes in diets, although sometimes it can be the result of infection or even stress (Isolauri et al., 2002). All these situations are usually encountered in animal production. When this imbalance occurs, it leads to an increase in inflammation in the intestinal tract and the enteric bacteria population is in state of flux (Huyghebaert et al., 2011). Niewold (2007) reported that gut inflammatory status has an effect on how AGPs impact on the microflora. The AGPs cannot add any benefits to the birds directly via an antimicrobial effect since they are provided at very low doses. The microflora of the intestinal tract consists of a large variety of microbial, and research has indicated that as the animals age the microbial population experiences a great deal of change, becoming more and more sophisticated (Lü et al., 2003). It becomes unlikely that a lone AGP could show consistent positive growth responses in such conditions; therefore a range of combination products are required.

Nieword (2007) also indicated that a number of popular AGP are in a category that accumulates in phagocytes with known attenuation of the innate inflammatory. This hypothesis is consistent

with the observation that the intestinal wall of animals fed AGPs tend to have thinner intestinal walls, which could be attributed to a reduced influx and accumulation of inflammatory cells (Jukes et al., 1956). AGPs have a minimal effect on the intestinal microbiota population, especially in the cecum (Reti et al., 2013). Even though some differences have been observed in the microbial population of the ileum between antibiotic-free birds and AGP-fed ones, it could have been affected by other diet factors and could contribute to the rapidly changing microbial population dynamics of the GIT (Wise & Siragusa, 2007).

2.6 The use of zinc-bacitractin as a growth promoter

Zinc-bacitracin (ZnB) is a mixture of high molecular weight polypeptides (bacitracin A, B and C and various minor components), first described in 1945 as a product of a Bacillus sp. (now regarded as Bacillus licheniformis). Bacitracin has several verified biological activities that affect microbial populations of the gut, and may account for the antibiotic's growth-promoting capability (Lin et al., 2009). The primary site of antibiotic activity is inside the GI tract, where ZnB acts to modify the intestinal flora as well as the gut structure (Visek, 1978; Stutz et al., 1983; Valfre, 1983; Armstrong, 1986; Boorman, 1987; Bernsten, 1994). However, the mechanisms of action of ZnB are not yet fully understood.

2.7 The ban of antibiotic growth promoter

One of the first countries to ban the use of AGP in livestock production, on account of the danger they pose to human health, was Sweden in 1986. In 1995, Denmark banned the use of avoparcin and glycopeptide antibiotics in diets of livestock. The EU also banned avoparcin in 1997 due to its cross-resistance with the vancomycin antibiotic. The streptogamin class of antibiotic such as virginiamycin, was banned in 1998 in Denmark because of possible cross-resistance with the human streptogamins such as quinupristin. In 1999, the EU banned a number of antibiotics (tylosin, virginiamycin, spiramycin and ZnB) in livestock production, but continued the use of bambermycin and avilamycin until the banning of all antibiotics in 2006 (Burch, 2006).

2.8 Antibiotic resistance in bacteria

Resistance to antibiotics related with the use of antibiotics in animals is an issue of principal concern. There are worries about the risk of transferring antibiotic-resistant pathogens via the food

chain and of transferring antibiotic-resistant genes from animal enteric flora to human pathogens. Secondly, there is a problem of decreased efficacy of antibiotics remedy in animals colonised with resistant bacteria. There is little research on antibiotic resistance in the bacterial isolates from animals. In spite of that, on account that the issues arose pertaining to AGP choice for resistance in human pathogens, a number of nations have set up antibiotic resistance surveillance programmes for bacterial isolates from animals (Barton, 2000; Martel & Coudert, 1993).

There is little research (Quesada et al., 2016; Liljebjelke et al., 2017) done on antibiotic resistance in E. coli and salmonella, as these bacteria are known as disease-causing micro-organisms in livestock. There is also very little statistics (Ndi & Barton, 2012) about antibiotic resistance in thermophilic campylobacter and enterococci, as these microorganisms are now not pathogenic; rather they are commensal enteric organisms. Antibiotic resistance can be seen in isolates of E. coli from animals soon after antibiotics were included into animal feeds (van den Bogaard et al., 2000). Studies in the UK in the late 1950s found that tetracycline resistance was already detectable in E. coli isolates from chickens and pigs fed rations containing much less than 100 g tetracycline/t (Smith, 1967). Resistance to different antibiotics used to be detected as new retailers had been introduced for therapeutic and growth-promotant purposes (Oluwasile et al., 2014; Smith, 1967; Anderson, 1968). Some researchers (Linton, 1986; Lee et al., 1993) also referred to the occurrence of tetracycline resistance, even in piggeries where tetracycline had not been used. It is commonly concluded that resistance persevered after antibiotics had been withdrawn (Smith, 1973; Rollins et al., 1976; Langlois et al., 1983; Hinton et al., 1984).

Antibiotic resistance in salmonella was also reported soon after animals started to be fed antibiotics at sub-therapeutic level (Anderson, 1968). As salmonella is a recognised food-borne pathogen, a range of the published reports of resistance patterns in animal isolates had been linked with studies

of human isolates or with concerns about resistance to specific antibiotics (Wray et al., 1986; Heurtin-Le Corre et al., 1999).

The mechanism of action of resistance development in antibacterial population happens when an antibiotic is applied in feed at sub-therapeutic level, which results in eliminating the sensitive population of bacteria, leaving the variants that have unusual traits and resist the effect (Apata, 2009). These resistant bacteria then multiply, becoming the predominant population. The resistant population so produced transmits the resistance genetically to subsequent progeny and to other bacterial strains via mutation or plasmid mediated (Catry et al., 2003). Humans are mainly exposed to such resistant bacteria through the consumption and handling of meat contaminated with such disease-causing microbes (Van den Bogaard & Stobberingh, 2000). The moment these resistant bacteria are acquired they colonise the intestinal tract of the human. The gene coding resistance to antibiotics in these bacteria can be transferred to more than a few microorganism belonging to the endogenous microflora of humans (Ratcliff, 2003; Stanton 2013), which lead to impediments in the effective treatment of bacterial infections.

2.9 Zootechnical additives as alternatives to antibiotic growth promoters in poultry

Zootechnical feed additives can be defined as any additive, which favours the overall performance of a healthy animal. They are four functional groups into which 'zootechnical additives' are categorized: digestibility enhancers, gut flora stabilizers, substance which favours the environment and other additives (Annex I of Regulation (EC) No 1831/2003).

2.9.1 Organic acids

Around the world, potential for poultry acidifiers has been rising due to the increasing demand for good quality poultry (Upadhayay & Vishwa, 2014). It has been proven that in some circumstances, organic acid can be used as an alternative to AGPs (Yadav et al., 2016). The foremost impact of antibiotics is antimicrobial; all their digestibility and overall performance effects can be explained by their impact on the gastrointestinal microflora. Like antibiotics, short chain organic acids have a specific antimicrobial activity (Kil et al., 2011). Unlike antibiotics, the antimicrobial activity of organic acids is pH-related (Eidelsburger et al., 1992; Boling et al., 2000;). Their antimicrobial activity is the result of the deleterious consequences of the free proton and, perhaps, the free anion on the bacterial cell (Dibner & Richards, 2005). Organic acids and their salts have been allowed as additives in poultry diets by the EU and have been declared safe (Adil et al., 2010).

Researchers (Brake, 2012; SA et al., 2008) additionally advise that in the intestine enterocytes, the dissociation of organic acids result in the synthesis and secretion of a hormone that subsequently stimulates pancreatic secretion. Thus, organic acids are known to have benefits beyond just antimicrobial activity. As for AGPs, the enhanced digestibility and overall performance effects

can be explained by their impact on the gastrointestinal microflora and the ensuing reduction in immune stimulation (Hermans & De Laet, 2014). Correspondingly, feeding organic acids reduces gram positive species of bacteria, with specific effect against acid-intolerant species like E. coli, Salmonella spp. and Campylobacter spp. (Hermans & De Laet, 2014). Both antibiotics and organic acids enhance protein and energy digestibility by lowering the immune stimulation and the ensuing synthesis and secretion of immune mediators (Dibner & Richards, 2005). Additionally, they both minimize the production of ammonia and different growth-depressing microbial metabolites and, perhaps, reduce the universal microbial load (Dibner & Richards, 2005)

A mixture of organic acids (Hassan et al., 2010; Hamed & Hassan, 2013) is reported to have a more synergistic effect with better efficiency than AGPs against intestinal colonised pathogens such as E. coli and salmonella. Organic acids have the potential to reduce the contamination of a litter with pathogens and diminish the risk of reinfection, thus reducing the bacterial challenge to poultry birds.

Organic acids such as carboxylic acids and fatty acids have a chemical formula of R COOH, where R represents the chain length of the acids. In poultry production, short chain organic acids like formic (C1), acetic (C2), propionic (C3) and butyric (4) have been used as feed additives more often (Dibner & Buttin, 2002). Other carboxylic acids that are used include citric, lactic, fumaric, malic and tartaric acids (Dibner & Buttin, 2002). Most organic acids are weak acids that dissociated partially in solution. The organic acids that possess antimicrobial activity have pKa values in the range of 3 to 5 (Khan & Iqbal, 2016)

2.9.2 Mode of action of organic acids in poultry gut

After ingestion, direct antimicrobial activity is greatest in the foregut, which has a very limited capability to change the digesta pH. Organic acids actively reduce microbial load, especially Escherichia coli and other acid-intolerant organisms (Khan & Iqbal, 2016). Many of these pathogens such as campylobacter and salmonella are opportunistic. A consequent reduction in subclinical infections may contribute to improved nutrient digestibility and a reduction in nutrient demand by the gut-associated immune tissue. The relatively low pH of the upper gut tends to favour not only the antimicrobial activity of organic acid, but also their absorption by diffusion into the gut epithelium (Mroz, 2000). Antimicrobial action in the crop is an important part of the organic acid benefit as it is a major site of colonisation for E.coli and salmonella. It is highly desirable for the organic acid activity to continue into the lower gut, where many of the anaerobic opportunistic pathogens are found. Lower microbial proliferation in the ileum is also important because it reduces the microflora's competition with the host for endogenous nitrogen lost into the gut by pancreatic and epithelial secretions and by enterocyte attrition and shedding. Continuous organic acid antimicrobial activity into the jejunum and ileum is also critical to another of its mechanisms of action. Lower microbial proliferation in the jejunum reduces the competition of the micro flora with the host for nutrients. This reduction in competition is the mechanism through which digestibility is improved (Huyghebaert et al., 2011).

There is a body of literature on the influence of organic acids on poultry production. Several experiments have been performed using fumaric acid. Vogt et al. (1981) reported on the impact of fumaric acids in broilers and layers. Fumaric acids improved feed efficiency by 3.5 to 4% in broilers while feed efficiency was also improved without the rate of lay being affected. Skinner et al. (1991) indicated a significant improvement in the weight gain of broilers with the use of 0.5 to

1% fumaric acid, but there was no impact on feed use. Very high acids have been associated with reduction in feed intake (FI) and body weights. Skinner et al. (1991) found a significant improvement of 49 d body weight and feed utilisation in male broilers fed 0, 0.125, or 0.5% fumaric acid. Dressing percentage, abdominal fat share and mortality were not affected. A similar study was reported by Dibner and Buttin (2002) in which 0.25 to 1% fumaric acid was compared to an AGP (Nitrovin) fed to Hubbard broilers. Feed consumption decreased while growth was not affected, resulting in a significant improvement in feed to gain. Feed efficiency for 0.5, 0.75 and 1 % fumaric acid have been comparable to the antibiotic control (Dibner & Buttin, 2002). Along with this performance improvement was a significant improvement in apparent metabolisable energy that was dose related. The linear regression analysis indicated an increase of 782.408 j/kg for each 1% of fumaric acid added.

Experiments additionally examined the overall performance outcomes of feeding malic, propionic, sorbic, tartaric, lactic and formic acid. The impact of buffered propionic acid, both in the presence and absence of ZnB, were discovered by Izat et al. (1990), who found a considerable increase in dressing proportion for female broilers and a large reduction in abdominal fat from adult males at 49d. There have been no difference performance results seen. Vogt et al. (1981), appeared at malic and tartaric acid (0.5 to 2%) in broilers. They found an increase in body weight achieve with foremost tiers of 1.12 and 1/3 for sorbic and tartaric acid, respectively. Sorbic and malic also showed signs of improving feed efficiency. Simons et al. (1990) observed the effect of dietary lactic acid on the overall performance of broilers at zero to 6 weeks of age. Body weight gains (BWG) tended to be greater, whereas feed-to-grain rations were significantly improved when birds were fed 2% lactic acids.

2.9.3 Minerals

Trace minerals such as zinc, copper, and manganese are required for a number of physiological processes in all animals. Livestock diets are mainly supplemented with minerals to prevent deficiencies which can lead to a number of clinical and pathological disorders. They play a vital role in a wide variety of body processes such as proper growth and development of all livestock animals. Trace minerals also act as a catalyst in both hormone and enzyme systems (Underwood & Suttle. 1999), which will directly have an impact on bone development, feathering, overall growth performance and appetite. Deficiencies can be caused by low mineral intake or by the presence of antagonists in the diet, which interfere with or lead to unbalance mineral uptake.

2.9.3.1 Zinc

Richards and Augustine, (1988) stated that Zn is one of the most vital trace elements and plays three main biological roles in the animal: structural, catalyst or regulatory. Zn is usually given in large quantities, more than required in the swine industry, for its growth permitting properties (Richards & Augustine, 1988), but in the broiler industry it is given according to the maintenance levels. The amount of Zn required for maintenance and growth, normally supplemented in broiler diets, is 0.012% to 0.018% (Nutr. Req. Polt., 1994). The inclusion rate of Zn for broilers, according to the National Research Council (NRC year 1994), is 40 ppm. The two main forms of Zn used in diets are zinc sulphate and zinc oxide. Additionally, Zn is a vital cofactor in a variety of biological pathways (Shankar & Prasad, 1998; Sahin et al., 2009). It plays a huge role as a cofactor in a number of enzymatic activities, including DNA and RNA synthesis (Shankar & Prasad, 1998). It also has the capability of interacting with bacteria and has a microbicidal effects in the gut.

Zinc deficiency in broiler diets can lead to increased susceptibility to infection (Keen & Gershwin, 1990) and will result in an inadequate growth and tissue accretion. Zn takes part in regulating immunity in cells by helping with leukocytes synthesis and phagocytosis (Sahin et al., 2009). Therefore, Zn deficiency may suppress the bird's immunity through diminished immune cell synthesis. Previous research indicated a diminished immune capacity in animals fed a Zn-deficient diet (Bao et al., 2003; Bao et al., 2006; Beck et al., 2006). Excessive Zn amounts can result in considerable harm to broiler health, even though broilers can generally tolerate the high amounts of Zn. Dewar et al. (1983) indicated a diminished growth and mortality in birds fed 2000, 4000 and 6000 ppm Zn from zinc oxide (Dewar & Downie, 1984). The broiler birds in the same experiment showed liver and pancreatic lesions. Bafundo et al. (1984) observed similar results with reductions in weight gain and feed efficiency. Other experiments indicated an excess of Zn in the kidney, liver and pancreas with supplementation of zinc oxide between 2000 and 5000 ppm (Blalock & Hill, 1988; Lü and Combs, 1988). The mode of action behind Zn supplementation is not yet fully understood, despite a large number of experiments done on the mineral.

In humans, Zn is commonly used in third-world countries and is administered to children to improve growth (Martorell, 2002). Zinc oxide is usually added to piglets' starter diets just after weaning to reduce diarrhoea (Katouli et al., 1999). Experiment on supplementation of zinc oxide indicates a significant reduction of diarrhoea in weaning piglets, but does not reduce the amount of E.coli excreted through faeces (Jensen-Waern et al., 1998). This suggests that most effect of Zn may be more pronounced in the intestine compared to the immune system. Studies on Zn supplementation indicates that it can minimise intestinal damage from a bacterial challenge (Zhang et al., 2012) and alleviate oxidative damage from a pathogenic challenge (Georgieva et al., 2011). These results may be due to Zn's antimicrobial properties. In vitro experiments of Zn showed that

Zn inhibits bacterial growth (Söderberg et al., 1990; Podbielski et al., 2000; Sawai, 2003). It was also noticed that zinc oxide supplementation has a greater effect on gram-positive bacteria (Söderberg et al., 1990), and gram-positive bacteria are more pathogenic compared to gram negative because they cause greater infection and resistance in the broiler industry (Nandi et al., 2004). However, further experiments using in vivo approaches must be done to confirm Zn's antimicrobial effects.

The relationship between Zn and the immune system is not well known in broilers. Roselli et al. (2003) indicated that zinc oxide do not reduce the number of colony-forming units cultured in media when added to plated E. coli. This suggests that zinc oxide does not have a direct effect on certain bacterial populations. However, it does not rule out the fact that zinc oxide has the potential to prevent bacterial adhesion to the intestinal wall (Roselli et al., 2003), maintaining intestinal integrity for improved feed efficiency and digestion. The supplementation of zinc oxide also reduces the inflammatory cytokine expression in vitro (Roselli et al., 2003), suggesting that zinc oxide has a positive effect on the immune system as well as intestinal morphology. The mode of action of this effect is not yet fully understood. Further in vivo studies are required to confirm the antimicrobial activity of Zn. Most experiments examining Zn supplementation in broilers have focused on mineral accretion in organs resulting from zinc toxicity (Bafundo et al., 1984; Lü & Combs, 1988; Blalock & Hill, 1988). Very few studies have focused on the potential benefits of zinc oxide in broiler chickens.

2.9.3.2 Manganese

Manganese is very vital for growth and fertility in animals. It is crucial for embryonic and post-natal bone development of the growing chick (Richards et al., 2010). It also activates enzymes in

the metabolism of carbohydrates, fats, proteins and nuclei acids. Manganese assist in collagen formation process, bone growth, urea formation and the functioning of the immune system (Zimbro & Power, 2003). It is also involved in enzymes associated with oxidative phosphorylation in mitochondria. The fundamental substance of the growing bone, in particular the proteoglycan matrix in which collagen and elastin are embedded, requires Mn for glycosylation of its protein core molecule (Bloom & Fawcett, 1997).

2.9.3.3 Copper

Copper is an essential mineral for the suitable maintenance and organ function of broiler chickens. It is also essential for reproduction and embryonic development and plays an important role in the proper cross-linking of collagen and elastin (Vieira, 2007). The dietary component requirements for copper are 8 mg/kg (Acetoze, 2013), and is mostly added in excess of requirements to improve broiler growth (Pesti & Bakalli, 1996; Ewing et al., 1998). Usually, Cu is supplemented in the forms of copper sulphate or copper chloride and copper montmorillonite. Research has shown that Cu possess antimicrobial properties (Aarestrup & Hasman, 2004) and can influence intestinal health and nutrient absorption. Cu plays a vital role in a number of biochemical pathways that may contribute to its antimicrobial qualities. Cu is an essential component of the superoxide dismutase pathway (Bozkaya et al., 2001), which plays a part in preventing oxidative damage to cells; thus, it helps to combat low levels of infection causing reactive oxygen species in cells. It is also a necessary component of a number of enzymes that function in increasing structural strength, elasticity of connective tissues and blood vessels (Rucker et al., 1998; Bozkaya et al., 2001).

Since copper has been shown to possess antimicrobial activity, it has been used in food services for its antimicrobial qualities (Faúndez et al., 2004). Cu also plays position in collagen cross-

linking, which has a direct impact on skin, bone, tendon and intestinal strength. Experiments in fowl have indicated that bone breaking strength correlates strongly with the extent of collagen cross-linking (Rath et al., 1999). Cu has the ability to interact with radicals and form hydroperoxide radicals, which have a great toxicity to bacteria (Suwalsky et al., 1998). Therefore, Cu ions have the potential to directly inhibit bacterial growth in the gut. Cu is usually supplemented as part of the ration as a growth promoter in the broiler industry, at levels between 125 and 250 ppm (Pesti & Bakalli, 1996). However, different views have been raised over the optimum level of Cu to supplement as different experiments use varying levels and forms (Banks et al., 2004; Luo et al., 2005; Arias & Koutsos, 2006; Pang et al., 2009). The optimum dose of Cu for growth promotion is not yet accurately known.

The mode of action of Cu on the gut is not yet known, despite its supplementation in the broiler industry for growth purposes (Acetoze, 2013). A number of researchers have tried to elucidate the impact of Cu on the gut. Arias & Koutsos (2006) showed that CuSO4 and tribasic copper chloride included in the feed increases the carcass weights of birds compared to un-supplemented birds. The same experiment also demonstrated a decreased number of intestinal intraepithelial lymphocytes in birds when supplemented with Cu compared to un-supplemented birds. It was concluded that Cu can modulate the intestinal lining and positively affect nutrient availability. The low numbers of intraepithelial lymphocytes also suggest a diminished immune response that could benefit growth by allocating more nutrients to digestion and growth rather than inflammation.

Copper montmorillonite has been shown to increase bird weight gain and feed efficiency compared to un-supplemented (Ma & Guo, 2008). Birds supplemented with Cu produce higher levels of digestive enzymes such as protease, lipase, trypsin and amylase (Ma & Guo, 2008), which provides an explanation for the improved feed efficiency and weight gain. The inclusion of copper-bearing

montmorillonite has also been shown to improve intestinal morphology, including increased villus height and decreased crypt depth in the duodenum, jejunum and ileum (Xia et al., 2004; Ma & Guo, 2008). A longer villus height provides greater surface area, which improves overall digestion. Longer crypts are a sign of a higher level of tissue regeneration, suggestive of infection. Shorter crypts allow for more energy to be focused on weight increase rather than intestinal tissue regeneration. The results of this experiment indicated that supplemented Cu has a direct effect on intestinal morphology itself to improve feed efficiency and weight gain. Pang et al. (2009) indicated an increase in the similarity of ileal microbiota in broilers supplemented with varying levels of Cu compared to un-supplemented birds.

Despite the positive research on the impact of Cu on broiler performance, there is still an inconsistency of literature towards broiler Cu supplementation. Some studies indicate supplementing Cu beyond dietary requirements improved average daily gain of broilers and FI (Arias & Koutsos, 2006; Ma & Guo, 2008; Lü et al., 2010) and others found contrary results for the same parameters (Luo et al., 2005; Miles et al., 2005; Pang et al., 2009). This difference may be due to the types of copper (Cu Sulfate and tribasic Cu Chloride) and various amounts used, which led to confusion regarding Cu's true antimicrobial mode of action. Further research into Cu's protection mechanism and its different forms is necessary to fully comprehend the most effective method of supplementing Cu to broiler feed.

2.9.3.4 Issues with trace minerals in poultry

The NRC has played a vital role in coming up with standardised nutrient requirements for livestock animals. Since 1994, the NRC has not updated for poultry (NRC, 1994), which led to a debate about the nutrient accuracy requirements. Over the years, genetic improvements have

resulted in an increased growth rate at a very young age. Thus poultry has exhibited higher growth rates and improved feed efficiency that probably needs greater available nutrient requirements. Most broiler producers have opted to supplement certain minerals (Cu, Mn and Zn) in excess due to their potential growth-promoting effect. Most minerals were included in the diet for purposes other than to meet traditional requirements and thus their dosages are way above NRC recommendations. NRC (1994) copper requirement for broilers is 8 mg/kg; however, it has routinely been given at 125 to 250 mg/kg as copper sulphate in both broiler and turkey diets to protect the health of birds and increase growth. The supper-dosing of one mineral in the diet causes a shortage of other minerals due to the inter-relationships among the minerals (Underwood & Suttle, 1991). There is also a possibility that these minerals can form chelates with phytate in the rations, which decreases the optimum efficiency of phytase (Maenz et al., 1999; Ondracek et al., 2002; Banks et al., 2004). Excess minerals in the manure have also increased the environmental burden (Blanco-Penedo et al., 2006).

Pressure from Regulatory Authorities has arisen to minimise environmental contamination caused by animal production. Most of the trace minerals included in the feed are excreted and these extra trace minerals, specifically copper and zinc, result in serious environmental pollution constraints (Ferket et al., 2002). Nys et al. (2001) noted that 95 to 99% of all ingested trace minerals appear in the faeces. Environmentalists also raise concerns that excess trace mineral in animal feed pollutes surface and ground water, and oil. Excreta from animals and broilers that were fed high copper and zinc diets led to an accumulation of these minerals in the soil and did not migrate extensively to water (Ferket et al., 2002). However, the minerals can be removed by plant uptake but can lead to crop toxicity. When the zinc concentration in the soil is above 200 ppm, the activity of soil microflora is reduced (Ferket et al., 2002). Dozier et al. (2003) showed that decreasing the

zinc concentration in the diet of chickens from 120 to 40 mg/kg reduced zinc excretion by 50%, and decreasing copper supplementation from 12 to 4 mg/kg reduced copper excretion by 35%. These results supported the use of exogenous enzymes that aid in liberating minerals and other chelated nutrients from feed ingredients, as well as the use of organic sources of minerals since they are added to the feed at lower concentrations.

2.9.4 Enzymes

Enzymes are one of the many types of proteins in biological systems whose function is to catalyse biological reactions. They are involved in all anabolic and catabolic pathways of digestion and metabolism (Buhler et al., 2013). The use of enzymes has expanded dramatically in the past 20 years and possibly one of their most vital actions is in mediating the diversity and composition of microbial populations. Feed enzymes improve digestibility thus leading to increased gut health. The nutrients required for the multiplication of bacteria in the intestinal tract are derived largely from dietary components, which are either not easily digested by endogenous enzymes or are absorbed so slowly that the bacteria in host's digestive system compete for them. Therefore, the use of exogenous feed enzymes has a direct impact on the diversity and numbers of gut micro floral populations (Bedford et al., 2012).

To comprehend the role of exogenous enzymes, the need to understand the structure and function of endogenous enzymes is vital. Endogenous enzymes such as trypsin, chemotrypsin, carboxypeptidase and elastase are all synthesised in the pancreas as zymogens. Initially, all zymogens are in inactive forms. After moving to the small intestine, they are subjected to proteolytic cleavage reactions, which eventually convert them to the active state (McCleary, 2010). Trypsin enzymes play a role in degradation of dietary protein. It is one the three principal digestive

proteinases, the other two being pepsin and chymotrypsin (Tabata et al., 2017). During the digestion process, trypsin acts with other proteinases to break down dietary protein molecules to peptides and amino acids. Chymotrypsin is synthesised in the pancreas of birds and has also been isolated from bovine pancreas. It is an endopeptidase, which preferentially splits peptide bonds in which the carboxyl group is contributed by the aromatic amino acids (tryptophan, tyrosine and phenylalanine). Chymotrypsin additionally catalyses the hydrolysis of the bonds of esters and amides of aromatic amino acids as well as proteins and peptides. Pepsin is also more environmentally friendly in cleaving bonds involving the aromatic acids and is synthesised in an inactive structure (pepsinogen) in the stomach lining. Hydrochloric acid is produced through the gastric mucosa, which helps to convert the inactive state of the enzyme and to maintain acidity for pepsin function (Hanson, 2014).

Exogenous in-feed supplementation of protease, amylase and xylanase enzymes is designed to breakdown protein, non-starch polysaccharides (NSP) and starch leading to improved digestion in monogastric animals fed corn-soybean meal diets (Burnett, 1966). Xylanase is an overall descriptor for variety of enzymes whose mode of action is manifested through the reaction of the xylan part of NSP existing in dietary components (Paloheimo et al., 2010). Hydrolysis of soluble NSP, generally in wheat-based diets, leads to reduced viscocity and extended passage rate of the digesta. This, in turn, decreases microorganism proliferation within the gut, endogenous secretions and accelerates nutrient absorption and growth performance. Hydrolysis of insoluble NSP decreases the water holding capability (WHC) of the epithelial duct leading to an increased digesta flow and nutrient diffusion. Hydrolysis of insoluble NSP will in addition increase cell membrane porousness, resulting in elevated nutrient diffusion (Bedford & Schulze, 1998; Zanelle et al., 1999).

The main goal of including exogenous enzymes in poultry diets is improving the efficiency of nutrient utilization in feed ingredients. This happens through the following modes of action: (i) breaking down particular bonds not usually degraded by endogenous digestive enzymes, (ii) degradation of the antinutritive elements that limit the availability of nutrients, (iii) improved accessibility of nutrients to endogenous digestive enzymes, and supplementation of enzymes in young animals (Bedford & Schulze, 1998). Exogenous enzymes affect the GI tract through increasing the digestibility of complex molecules, especially in young animals that do not have a well-developed intestinal enzyme profile (Mahagna et al., 1995; Leeson & Summers, 2001). Research has indicated that the negative nutritional effects of non-starch polysaccharides (NSP), oligosaccharides and phytic acid can be overcome with the aid of supplementation of diets with suitable exogenous enzyme preparations (Adeola & Cowieson, 2011). Studies have indicated that the use of exogenous enzymes in cereal-based diets is very beneficial since it helps to hydrolyse NSP; reduce digesta viscosity and most importantly, it improves nutrient absorption, growth and performance of the birds (Iji, 1999; Hetland et al., 2004; Wang et al., 2005).

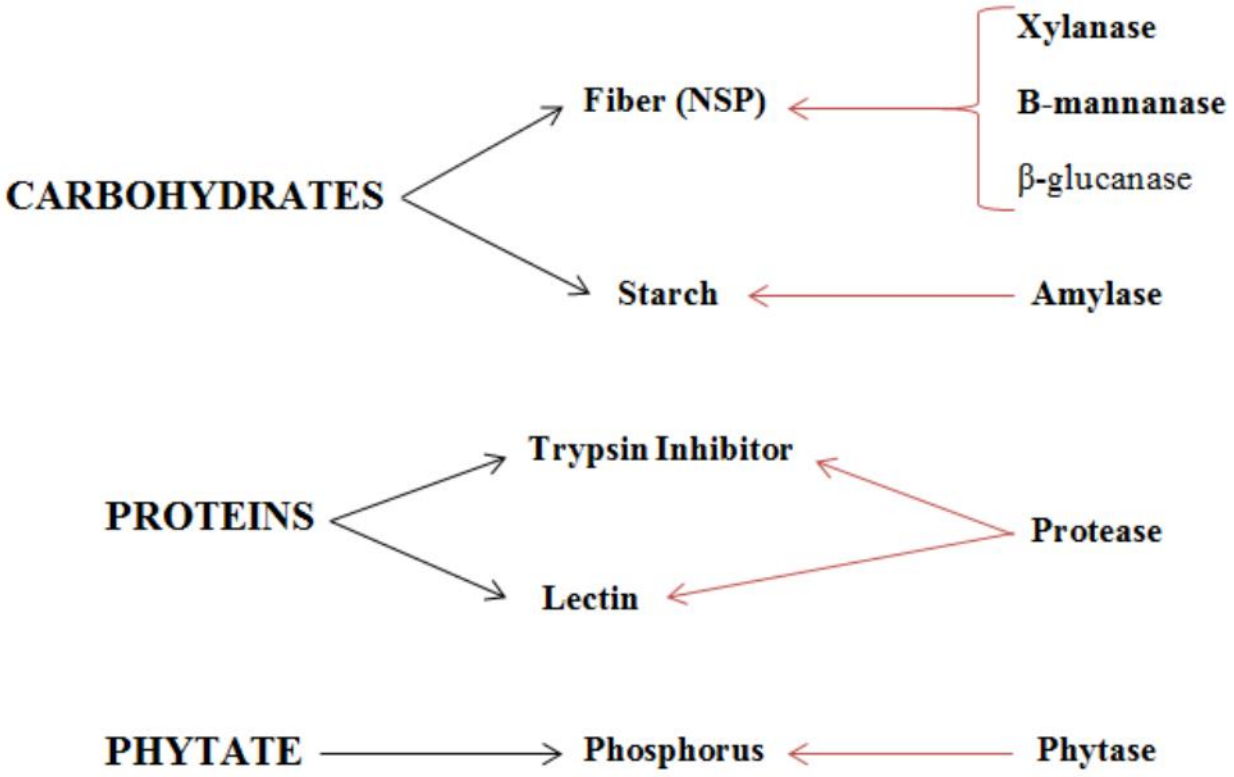

Figure 2.1: Substrates and anti-nutrients targeted by some commonly utilised exogenous enzymes. (Bedford & Partridge, 2010).

The mode of action by which feed enzymes impact the intestinal microbiota have been acknowledged for some time such as via enhanced nutrient delivery to the host and through provision of fermentable oligosaccharides. However, the extent to which this effect contributes to the net benefit of the enzyme use is still unknown, and it is not clear which major microbial species are involved (Bedford & Cowieson, 2012). Recent studies show that if feed enzymes are to substitute prophylactic antibiotics, then the objective of the enzymes must be to enrich the ileum and cecum with Clostridium cluster XIVa species and E. coli whilst depressing the numbers of Lactobacillus spp. (Czerwiński et al., 2010).

The use of exogenous enzymes in poultry feed is of great value. The ever-increase in the price of feed ingredients and banning of AGPs has been a major concern in most developing countries. Consequently, cheaper and non-antibiotic feed ingredients have to be used.

2.9.5 Effects of enzymes on the gastrointestinal environment

Micro-organisms in the GI tract use the digesta for energy in a similar manner to the host animal. Changes in rate of passage and the kind of nutrients available to the microbes have an impact on the distinct microbial populations in the GI tract. The end product of metabolism of many of the anaerobic bacteria observed in the gut are unstable fatty acids that have been proven to be altered with enzyme supplementation (Choct et al., 1995). However, research studies analysing variations in precise microbial populations such as starch or xylan-degrading microorganism has not yielded meaningful results (Persia et al., 1999). This may be due to lack of technology to adequately examine these populations since it stands to reason that as the substrate changes so should the micro-organisms that can use them. Gastrointestinal histology is affected by barley and wheat-based diets with reductions in villi height, increased diameter and damaged villi being observed (Viveros et al., 1994; Jaroni et al., 1999). Enzyme supplementation of these diets counteracted some of these results with supplemented birds having an intestine morphology similar to birds receiving a corn/soy diet. This can also help explain reduction in mortality often observed in birds receiving enzyme supplementation (Bedford & Morgan, 1996). Damage to the GI tract may make the organ extra inclined to pathogenic bacterial invasion. In addition, enzyme supplemented birds had lower intestine and pancreas weights (Bedford & Morgan, 1996). Enzymes continue to receive recognition among animal producers due to their capability to improve overall performance and reduce nutritional variation in feed ingredients.

2.9.6 Protease enzyme

Poultry feed consists of complex, ingestible compounds such as large globular proteins (Evans & Moritz, 2014). Protease may be included in the diet to increase crude protein and amino acid digestibility. Physiologically, proteases play an important role in cellular catabolism of digestion and protein turnover of the immune system (Barrett, 1995). Exopeptidases and endopeptidases are a sophisticated group of enzymes capable to hydrolyse the peptide bond in a protein molecule. Exopeptidases cleave the peptide bond proximal to the amino or carboxyl termini of the substrate, where endopeptidases cleave interior peptides bonds (McDonald et al 2011). In reference to the functional group existing at the active side, proteases are classified into four groups: serine, cysteine, aspartic and metalloprotease (Gupta et al., 2002).

Protease is a protein-digesting enzyme that breaks down storage proteins binding starch within feed ingredients. This makes the energy from protein-bound starch available to the bird to be used for productive purposes. Proteases are also effective in breaking down anti-nutrients, which are proteins found in ingredients like soybean meal.

The addition of protease enzymes has a number of advantages including enhancing protein digestion resulting in improved amino acid digestibility across various protein sources. Protease enzyme reduces the impact of anti-nutritional factors by degrading anti-nutritional factors and allergenic proteins in feedstuffs such as soya beans. It also supports gut health as it improves protein digestibility and reduces undigested protein entering the hind gut. Reduced protein fermentation in the large intestine improves gut health. This has been verified by the increased villus height to crypt depth ratio and decreased Clostridium perfringens proliferation (Vivoros et al., 2011).

The use of protease enzymes in poultry feed has a significant impact on gut health because undigested proteins in the hind gut have an adverse impact on gut health and necrotic enteritis risk.

2.9.7 Probiotics

Probiotics have been used in the poultry industry and they are quickly becoming accepted as a potential alternative to antibiotics for use as growth promoters and, in some cases, to control specific enteric pathogens (Huff et al., 2007) and improve the health status of an animal by maintaining a beneficial microbial population (Lee et al., 1993; Fuller, 2001). Probiotics are live micro-organisms, generally bacteria or yeast that, when ingested alive and in sufficient quantities, have a positive effect on health that goes beyond the common nutritional effects (Fuller, 1989). Their use is related to a proven efficacy on the gut microflora, resulting in improved health status. Micro-organisms that can be used in animal feed are mainly bacterial strains of gram-positive bacteria belonging to the types Bacillus (B. cereus var. toyi, B. licheniformis, B. subtilis), Enterococcus (E. faecium), Lactobacillus (L. acidophilus, L. casei, L. farciminis, L. plantarum, L. rhamnosus), Pediococcus (P. acidilactici) or Streptococcus (S. infantarius); some others probiotics are microscopic fungi such as strains of yeast belonging to the Saccharomyces cerevisiae species and kluyveromyces (Anadón et al., 2006). An ideal probiotic has the following characteristics: non-pathogenic, of host origin, able to withstand processing and storage, resists gastric acid and bile, adheres to epithelium or mucus, persists in the intestinal tract, produces inhibitory compounds, modulates immune respond and, finally, it must be able to alter microbial activities.

Probiotic supplementation will interfere with the flexibility of the infective bacterium to colonise the epithelial duct and infect the membrane (Gill, 2003). Therefore, alternative gut health stems from this method of competitive exclusion of infective bacterium (Nurmi & Rantala, 1973;

Schneitz, 2005), improving bird performances (Corrier et al., 1995) and immune functions (Schneitz, 2005).

The effects of probiotics on broiler performance, gut morphometry and immune parameters have been well-documented (Lee et al., 2010; Awad et al., 2008 & Salim et al., 2013). The maintenance of the mucosa lining of the GI tract is a key factor in preserving barrier function and animal immunity (Denbow, 2000; Gill, 2003). A non-supplemented mash diet was given to day-old broiler chicks from day 1 to day 2 and supplemented with probiotics from day 3 to day 22 (Lee et al., 2010). Eight differing strains of B. subtilis and one multistrain probiotic were fed (1.5 × 105 cfu/g probiotic) by Denbow, 2000. The results showed no significant alteration in body weight; however, intestinal morphology was altered by supplementation of probiotics. Significant increases in villus height and reduced serum levels of α-1-acid glycoprotein, an indicator of inflammation, were observed in probiotics-treated broilers compared to non-supplemented control. Villus height increases are associated with increased absorption capacity of nutrients (Denbow, 2000) and probiotics have also shown an increase in villus height in human gastrointestinal systems (Hooper et al., 2002). Probiotics may also be involved in the regulation of the hind gut fermentation and synthesis of short chain fatty acids, and can increase the efficiency of gut microbes to ferment NSP (Sekata, 1987).

In another study male broiler chicks were fed corn-soybean meal-based diets supplemented with a commercial probiotic (0, 0.2, or 0.6%) and antibiotic (0 or 6 ppm flavomycin) from 1 to 42 d (Li et al., 2008). From 1 to 21 d birds were fed with a starter diet and were then transitioned to grower diets from 22 to 42 d. The treatments supplemented with probiotics showed no significance in Body weight gain BWG or Feed intake (FI) during the 42 d period. However, probiotic inclusion consistently improved the apparent ileal digestibility of CP and amino acids. Probiotic

supplementation continued to show significant improved ileal digestibility of dry matter, energy, calcium, and phosphate. From these data, it was concluded that probiotics could be an effective alternative to antibiotics. Other studies have indicated that supplementation of probiotics in broiler diets improved performance, feed efficiency and BWG for broilers fed corn-soya meal diets (Jin et al., 1998) and for broiler chicks fed probiotics for 45 d.

2.9.7.1 Mechanism of action of probiotics

Nutritional effect is characterised by a reduction of metabolic reactions that produce toxic substances, stimulation of indigenous enzymes and production of vitamins and antimicrobial substances. Health or sanitary effect is distinguished by an increase in colonisation resistance, competition for gut surface adhesion and stimulation of the immune response (Guillot, 2003). The last effect acts as a bio-regulator of the gut microflora and reinforces the host's natural defence. The probiotic has a vital role in the balance of gut microflora, increasing the resistance to pathogenic agents, both through a strengthening of the intestinal barrier and directly stimulating the immune system.

2.10 Bone challenges in broilers

Poultry is one of the most popular food source animals throughout the world. In the U.S. alone, more than 9 billion broilers were hatched and reared in 2015 (NASS, 2016). In the past century and especially the last several decades, broilers have been successfully selected for a short growth cycle plus high meat yield, in particular breast muscle. However, the programme also makes the broilers more susceptible to metabolic disorders and leg abnormalities that lead to poor locomotion. Predominantly affecting the locomotor system and resulting in impaired mobility or lameness, leg disorders have become a considerable problem in commercial poultry meat

production. Leg disorders in broilers could be caused by multiple factors, such as abnormal bone development, infections and degenerative diseases (European Commission, 2000).

A common method for evaluating leg problems is assessing lameness. Two gait scoring systems have been developed to rate the lameness of broilers, either the 0 to 5 scale (Kestin et al., 1992; Garner et al., 2002) or the 0 to 2 scale gait score (Webster et al., 2008), with the lower score indicating better leg health. The latency-to-lie test is another method commonly used to assess lameness. This test was developed by Weeks et al. (2002) based on the fact that broilers are averse to be in contact with water, and was modified for simplicity and effectiveness by Berg and Sanotra (2003). The latency-to-lie test is highly and negatively correlated to gait score (Weeks et al., 2002; Berg & Sanotra, 2003).

The above literature has described the effects of zootechnical feed additives on growth promoters and they are no consistency in the studies, which call for more studies to be done. There is also limited information of the effect of the additives on other parameters such as blood and meat parameters. Combining zootechnical feed additives as an alternative approach to antibiotic growth promoters in broiler diets is not enough, which indicates that more studies are required to test different combinations since individual additives are not producing desirable results.

2.11 References

Aarestrup, F.M. & Hasman, H., 2004. Susceptibility of different bacterial species isolated from food animals to copper sulphate, zinc chloride and antimicrobial substances used for disinfection. Vet. Microbiol. 100(1), 83-89.

Acetoze, G., Kurzbard, R., Ramsey, J.J., Klasing, K.C. & Rossow, H.A., 2013. Influence of mitochondrial function on feed efficiency of broilers with and without growth enhancing levels of minerals supplementation during a coccidiosis challenge. In: Energy and protein metabolism and nutrition in sustainable animal production. Eds: Oltjen, J.W., Kebreab, E. & Lapierre, H., Wageningen Academic Publishers, The Netherlands, pp. 393-394.

Adeola, O. & Cowieson, A., 2011. Board-invited review, opportunities and challenges in using exogenous enzymes to improve non-ruminant animal production. J. Anim. Sci. 89(10), 3189-3218.

Adil, S., Banday, T., Bhat, G.A., Mir, M.S. & Rehman, M., 2010. Effect of dietary supplementation of organic acids on performance, intestinal histomorphology, and serum biochemistry of broiler chicken. Vet. Med. Int. 2010; 2010: 479485 doi: 10.4061/2010/479485

Amit-Romach, E., Sklan, D. & Uni. Z., 2004. Microflora ecology of the chicken intestine using 16S ribosomal DNA primers. Poultry Sci. 83(7), 1093-1098.

Anadón, A., Martínez-Larrañaga, M.R. & Martínez, M.A., 2006. Probiotics for animal nutrition in the European Union. Regulation and safety assessment. Regul. Toxicol. Pharm. 45(1), 91-95.

Anderson, E., 1968. Drug resistance in Salmonella typhimurium and its implications. Br. Med. J. 3(5614), 333-339.

Apajalahti, J., Kettunen, A. & Graham, H., 2004. Characteristics of the gastrointestinal microbial communities, with special reference to the chicken. World Poultry Sci. J. 60(2), 223-232.

Apajalahti, J., Kettunen, A., Bedford, M. & Holben, W., 2001. Percent G+C profiling accurately reveals diet-related differences in the gastrointestinal microbial community of broiler chickens. Appl. Environ. Microbiol. 67, 5656-5667.

Apata, D.F., 2009. Antibiotic resistance in poultry. Int J. Poultry Sci., 8(4), pp.404-408.

Arias, V. & Koutsos, E., 2006. Effects of copper source and level on intestinal physiology and growth of broiler chickens. Poultry Sci. 85(6), 999-1007.

Armstrong, D.G., 1986. Gut-active growth promoters. Control and Manipulation of Animal Growth: Proceedings of Previous Easter Schools in Agricultural Science, p.21.

Awad, W., Ghareeb, K. and Böhm, J., 2008. Intestinal structure and function of broiler chickens on diets supplemented with a synbiotic containing Enterococcus faecium and oligosaccharides. Int J. Mol Sci. 9(11), pp.2205-2216.

Bafundo, K.W., Baker, D.H. & Fitzgerald, P.R., 1984. The iron-zinc interrelationship in the chick as influenced by Eimeria acervulina infection. J. Nutr. 114(7), 1306-1312.

Banks, K., Thompson, K., Jaynes, P. & Applegate, T., 2004. The effects of copper on the efficacy of phytase, growth, and phosphorus retention in broiler chicks. Poultry Sci. 83(8), 1335-1341.

Bao, E.D., Gong, Y.Y., Hartung, J., Fu, X.B., Wang, X.J., Zhang, H.T. & Wang, Z. L., 2003. Relation between pathologic damages and HSP70 changes in acute heat stressed broilers. Sci. Agric. Sin. 37, 301-305.

Bao, S., Wu, Q., McLendon, R.E., Hao, Y., Shi, Q., Hjelmeland, A.B., Dewhirst, M.W., Bigner, D.D. and Rich, J.N., 2006. Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. Nature, 444(7120), 756.

Barnes, E.M., Impey, C.S. & Stevens, B.J.H., 1979. Factors affecting the incidence and anti-salmonella activity of the anaerobic caecal flora of the young chick. J. Hyg-Cambridge. 82, 263-283.

Barnes, E.M., Mead, G.C., Barnum, D.A. & Harry, E.G., 1972. The intestinal flora of the chicken in the period 2 to 6 weeks of age, with particular reference to the anaerobic bacteria. Brit. Poultry Sci. 13, 311-326.

Barrett, S., 1995. Heterogeneous international environmental agreements. Milano: Fondazione ENI Enrico Mattei.

Barton, L., 2000. Crisis in organizations II. South-Western College Publishing.

Beck, F.W., Li, Y., Bao, B., Prasad, A.S. & Sarkar, F.H., 2006. Evidence for reprogramming global gene expression during zinc deficiency in the HUT-78 cell line. Nutr. J. 22(10), 1045-1056.

Bedford, M. & Schulze, H., 1998. Exogenous enzymes for pigs and poultry. Nutr. Res. Rev. 11(1), 91-114.

Bedford, M.R. & Cowieson, A.J., 2012. Exogenous enzymes and their effects on intestinal microbiology. Anim. Feed Sci. Tech. 173(1-2), 76-85.

Bedford, M.R. & Partridge, G.G., 2010. Enzymes and farm animal nutrition. 2nd edn. CABI. Cambridge, England.

Bedford, M.R. and Morgan, A.J., 1996. The use of enzymes in poultry diets. World's Poultry Sci` J. 52(1), pp.61-68.

Bedford, M.R., Ghosh, T.K., Haldar, S., Muthusami, N. & Samanta, I., 2012. Assessment of yeast cell wall as replacements for antibiotic growth promoters in broiler diets: effects on performance, intestinal histo-morphology and humoral immune responses. J. Anim. Physiol. Anim. Nutr. 96(2), 275-284.

Berg, R.D., 1996. The indigenous gastrointestinal microflora. Trends Microb. 4(11), 430-435.

Berntsen, D., 1994. Involuntary autobiographical memories. Appl. Cognitive Psych. 10(5), 435-454.

Blalock, T.L. & Hill, C., 1988. Studies on the role of iron in zinc toxicity in chicks. Biol. Trace Elem. Res. 17(1), 17-29.

Blanco-Penedo, I., Cruz, J.M., Lopez-Alonso, M., Miranda, M., Castillo, C., Hernández, J. & Benedito, J.L., 2006. Influence of copper status on the accumulation of toxic and essential metals in cattle. Environ. Int. 32, 901-906.

Boling, S.D., Webel, D.M., Mavromichalis, I., Parsons, C.M. & Baker, D.H., 2000. The effects of citric acid on phytate-phosphorus utilization in young chicks and pigs. J. Anim. Sci. 78(3), 682-689.

Boorman, K. N., 1987. Mode of action of gut-active (antibiotic) performance promoters. In: Proceedings of the Sixth European Symposium on Poultry Nutrition. Editor: H. Vogt, World's Poultry Science Association, Celle, Germany, pp. D12–20

Bozkaya, L.A., Öztürk-Ürek, R., Aydemir, T. & Tarhan, L., 2001. Effects of Se, Cu and Se+ vitamin E deficiency on the activities of CuZnSOD, GSH-Px, CAT and LPO levels in chicken erythrocytes. Cell. Biochem. Funct. 19(3), 153-157.

Brake, D.W., 2012. Effects of duodenal amino acid infusion on small intestinal starch digestion in cattle (Doctoral dissertation, Kansas State University).

Bray, J.L., Taylor, C.S., Cherry, T.E. & Carey, J., 2008. Performance comparison between the use and nonuse of an enteric health medication program across five consecutive commercial broiler flocks. J. Appl. Poultry Res. 18(2), 165-171.

Buhler, K., C. Wenk, J. Broz, and S. Gebert. 2006. Influence of benzoic acid and dietary protein level on performance, nitrogen metabolism and urinary pH in growing-finishing pigs. Arch. Anim. Nutr. 60, 382-389.

Burch, D. 2006. Anticipated effects of the withdrawal of antibiotic growth promoters (AGPs) from pigs in the European Union on 1st January 2006. http://www.octagon-services. co.UK/articles/withdrawalAGP.htm. Accessed Aug. 2006.

Burnett, G., 1966. Studies of viscosity as the probable factor involved in the improvement of certain barleys for chickens by enzyme supplementation. Brit. Poultry Sci. 7(1), 55-75.

Casewell, M., Friis, C., Marco, E., McMullin, P. and Phillips, I., 2003. The European ban on growth-promoting antibiotics and emerging consequences for human and animal health. J. Antimicrob. Chemoth. 52(2), 159-161.

Castanon, J.I., 2007. History of the use of antibiotic as growth promoters in European poultry feeds. Poultry Sci. 86(11), 2466-2471.

Catry, B., Laevens, H., Devriese, L.A., Opsomer, G. & Kruif, A.D., 2003. Antimicrobial resistance in livestock. J. Vet. Pharmacol. Ther. 26(2), 81-93.

Choct, M., Hughes, R.J., Wang, J., Bedford, M.R., Morgan, A.J. & Annison, G., 1995. Feed enzymes eliminate the antinutritive effect of non-starch polysaccharides and modify fermentation in broilers. In: Proceedings of the Australian Poultry Science Symposium. 7, 121-125.

Ciampolini, M., Bini, S. & Orsi, A., 1996. Microflora persistence on duodenojejunal flat or normal mucosa in time after a meal in children. Physiol. Behav. 60(6), 1551-1556.

Corrier, D.E., Nisbet, D.J., Scanlan, C.M., Hollister, A.G., Caldwell, D.J., Thomas, L.A., Hargis, B.M., Tomkins, T. & Deloach, J.R., 1995. Treatment of commercial broiler chickens with a characterized culture of cecal bacteria to reduce salmonellae colonization. Poultry Sci. 74(7), 1093-1101.

Czerwiński, J., Højberg, O., Smulikowska, S., Engberg, R.M. & Mieczkowska, A., 2010. Influence of dietary peas and organic acids and probiotic supplementation on performance and caecal microbial ecology of broiler chickens. Brit. Poult. Sci. 51(2), 258-269.

Denbow, D.M., 2000. Gastrointestinal anatomy and physiology. In: Sturkie's Avian Physiol. Ed: Whittow, G.C., New York Academic Press, pp. 299–325.

Dewar, W. & Downie, J., 1984. The zinc requirements of broiler chicks and turkey poults fed on purified diets. Brit. J. Nutr. 51(3), 467-477.

Dewar, W., Wight, P., Pearson, R.A. & Gentle, M., 1983. Toxic effects of high concentrations of zinc oxide in the diet of the chick and laying hen. Brit. Poultry Sci. 24(3), 397-404.

Dibner, J.J & Richards, J.D., 2005. Antibiotic growth promoters in agriculture: history and mode of action. Poultry Sci. 84(4), 634-643.

Dibner, J.J. & Buttin, P., 2002. Use of organic acids as a model to study the impact of gut microflora on nutrition and metabolism. J. Appl. Poultry Res. 11(4), 453-463.

Dozier Iii, W.A., Davis, A.J., Freeman, M.E. and Ward, T.L., 2003. Early growth and environmental implications of dietary zinc and copper concentrations and sources of broiler chicks. Brit. Poultry Sci. 44(5), 726-731.

Eidelsburger, U., Kirchgessner, M. & Roth, F.X., 1992. Zum Einfluß von Fumarsäure, Salzsäure, Natriumformiat, Tylosin und Toyocerin auf tägliche Zunahmen, Futteraufnahme, Futterverwertung und Verdaulichkeit. J. Anim. Physiol. Anim. Nutr. 68(2), 82-92.

Elwinger, K.A., Schneitz, C., Berndtson, E., Fossum, O., Teglöf, B. & Engstöm, B., 1992. Factors affecting the incidence of necrotic enteritis, caecal carriage of Clostridium perfringens and bird performance in broiler chicks. Acta Veterinaria Scandinavica 33(4), 369-378.

Engberg, R.M., Hedemann, M.S., Leser, T.D. & Jensen, B.B., 2000. Effect of zinc bacitracin and salinomycin on intestinal microflora and performance of broilers. Poultry Sci. 79, 1311-1319.

Evans, A.M., Loop, S. A., & Moritz, J.S., 2014. The effect of poultry litter biochar on pellet quality, 1-21d broiler performance. J. Appl. Poult. Res. 24:380–386

Ewing, H.P., Pesti, G.M., Bakalli, R.I. & Menten, J., 1998. Studies on the feeding of cupric sulfate pentahydrate, cupric citrate, and copper oxychloride to broiler chickens. Poultry Sci. 77(3), 445-448.

Faúndez, G., Troncoso, M., Navarrete, P. & Figueroa, G., 2004. Antimicrobial activity of copper surfaces against suspensions of Salmonella enterica and Campylobacter jejuni. BMC. Microbiol. 4(1), 19

Fawcett, D.W. & Jensh, R.P., 1997. Concise histology. Chapman and Hall.

Ferket, P.R., Parks, C.W. & Grimes, J.L., 2002. Benefits of dietary antibiotic and mannan oligosaccharide supplementation for poultry. In: Multi-State Poultry Meeting Vol. 14.

Finegold, S.M., Flora, D.J., Attebery, H.R. and Sutter, V.L., 1975. Fecal bacteriology of colonic polyp patients and control patients. Cancer Res. 35, 3407-3417.

Fuller, R., 1989. Probiotics in man and animals. J. Appl. Bacteriol. 66, 365-378.

Fuller, R., 2001. The chicken gut microflora and probiotic supplements. J. Poultry Sci. 38(3), 189-196.

Garner, J.P., Falcone, C., Wakenell, P., Martin, M. and Mench, J.A., 2002. Reliability and validity of a modified gait scoring system and its use in assessing tibial dyschondroplasia in broilers. Brit. Poultry Sci. 43(3), 355-363.

Georgieva, N.V., Gabrashanska, M., Koinarski, V. & Yaneva, Z., 2011. Zinc supplementation against Eimeria acervulina – induced oxidative damage in broiler chickens. Vet. Med. Int. 2011, 1 – 8.

Gibson, U.E., Heid, C.A. and Williams, P.M., 1996. A novel method for real time quantitative RT-PCR. Genome research, 6(10), .995-1001.

Gill, K., 2003. A comparison of two culture media for the recovery of thermophilic Campylobacters in broiler farm samples. J. Microb. Methods, 54(3), 367-371.

Graham, I.D. & Tetroe, J., 2007. CIHR research: How to translate health research knowledge into effective healthcare action. Healthcare Quarterly, 10(3).

Gupta, R., Beg, Q. & Lorenz, P., 2002. Bacterial alkaline proteases: molecular approaches and industrial applications. Appl Microbiol Biotech, 59(1), pp.15-32.

Hamed, D.M. & Hassan. A.M.A., 2013. Acids supplementation to drinking water and their effects on Japanese quails experimentally challenged with Salmonella enteritidis. Zool. Res. 3, 15-22.

Hanson, M.N., 2014. Effects of exogenous enzymes and direct-fed microbial on broiler performance and nutrient digestibility when fed variable inclusions of soy products. Doctoral dissertation, Iowa State University.

Hassan, H.M.A., Mohamed, M.A., Youssef, A.W. & Hassan, E.R., 2010. Effect of using organic acids to substitute antibiotic growth promoters on performance and intestinal microflora of broilers. Asian-Australas. J. Anim. Sci. 23(10), 1348-1353.

Hermans, D. & De Laet, M., 2014. Reaching genetic potential with medium chain fatty acids (MCFAs). Int. Poultry Prod. 22, 7-9.

Hetland, H., Choct, M. & Svihus, B., 2004. Role of insoluble non-starch polysaccharides in poultry nutrition. World Poultry Sci. J. 60(4), 415-422.

Heurtin-Le Corre, C., Donnio, P-Y., Perrin, M., Travert, M-F. & Avril, J-L., 1999. Increasing incidence and comparison of nalidixic acid-resistant salmonella enterica subsp. enterica serotype typhimurium isolates from humans and animals. J. Clin. Microbiol. 37(1), 266-269.

Hinton, M., Rixson, P., Allen, V. & Linton, A., 1984. The persistence of drug-resistant Escherichia coli strains in the majority faecal flora of calves. Epidemiol. Infect. 93(3), 547-557.

Hoerr , F. J. 1998. Pathogenesis of enteric diseases. Poult. Sci. 77 : 1150 –1155.

Hofacre, C.L. Lü, J. & Lee, M.D., 2006. Emerging technologies in microbial ecology aid in understanding the effect of monensin in the diets of broilers in regard to the complex disease necrotic enteritis. J. Appl. Poultry Res. 15(1), 145-153.

Hofacre, C.L., Froyman, R., Gautrias, B., George, B., Goodwin, M.A. & Brown, J., 1998. Use of Aviguard and other intestinal bioproducts in experimental Clostridium perfringens-associated necrotizing enteritis in broiler chickens. Avian Diseases, pp.579-584.

Hooper, L.V., Midtvedt, T. & Gordon, J.I., 2002. How host-microbial interactions shape the nutrient environment of the mammalian intestine. Annu. Rev. Nutr. 22(1), 283-307.

Huff, G., Huff, W., Rath, N., De Los Santos, F.S., Farnell, M. & Donoghue, A., 2007. Influence of hen age on the response of turkey poults to cold stress, Escherichia coli challenge, and treatment with a yeast extract antibiotic alternative. Poultry Sci. 86(4), 636-642.

Huyghebaert, G., Ducatelle, R. & Van Immerseel, F., 2011. An update on alternatives to antimicrobial growth promoters for broilers. Vet. J. 187(2), 182-188.

Iji, P., 1999. The impact of cereal non-starch polysaccharides on intestinal development and function in broiler chickens. World Poultry Sci. J. 55(4), 375-387.

Isolauri, E., Kirjavainen, P.V. and Salminen, S., 2002. Probiotics: a role in the treatment of intestinal infection and inflammation?. Gut, 50(suppl 3), pp.iii54-iii59.

Izat, A.L., Tidwell, N.M., Thomas, R.A., Reiber, M.A., Adams, M.H., Colberg, M. & Waldroup, P.W., 1990. Effects of a buffered propionic acid in diets on the performance of broiler chickens and on microflora of the intestine and carcass. Poultry Sci. 69(5), 818-826.

Jaroni, D., Scheideler, S.E., Beck, M. & Wyatt, C., 1999. The effect of dietary wheat middlings and enzyme supplementation. 1. Late egg production efficiency, egg yields, and egg composition in two strains of Leghorn hens. Poultry Sci. 78(6), 841-847.

Jensen-Waern, M., Melin, L., Lindberg, R., Johannisson, A., Petersson, L. & Wallgren, P., 1998. Dietary zinc oxide in weaned pigs – effects on performance, tissue concentrations, morphology, neutrophil functions and faecal microflora. Res. Vet. Sci. 64(3), 225-231.

Jin, L.Z., Ho, Y.W., Abdullah, N., Ali, M.A. & Jalaludin, S., 1998. Effects of adherent Lactobacillus cultures on growth, weight of organs and intestinal microflora and volatile fatty acids in broilers. Anim. Feed Sci. Tech. 70(3), 197-209.

Jukes, T.H., Hill, D. & Branion, H., 1956. Effect of feeding antibiotics on the intestinal tract of the chick. Poultry Sci. 35(3), 716-723.

Jukes, T.H., Stokstad, E.L.R., Taylor, R.R., Combs, T.J., Edwards, H.M. & Meadows, G.B., 1950. Growth-promoting effect of aureomycin on pigs. Arch. Biochem. 26, 324-330.

Katouli, M., Melin, L., Jensen-Waern, M., Wallgren, P. & Möllby, R., 1999. The effect of zinc oxide supplementation on the stability of the intestinal flora with special reference to composition of coliforms in weaned pigs. J. Appl. Microbiol. 87(4), 564-573.

Keen, C.L. & Gershwin, M.E., 1990. Zinc deficiency and immune function. Ann. Rev. Nutr. 10(1), 415-431.

Kestin, S.C., Su, G.& Sorensen, P., 1999. Different commercial broiler crosses have different susceptibilities to leg weakness. Poultry Sci. 78(8), 1085-1090.

Khan, S.H. & Iqbal, J., 2016. Recent advances in the role of organic acids in poultry nutrition. J. Appl Anim Res, 44(1), pp.359-369.

Khan, S.H. and Iqbal, J., 2016. Recent advances in the role of organic acids in poultry nutrition. J. Appl Anim Res, 44(1), pp.359-369.

Kil, D.Y., Han, G.G., Kim, E.B., Lee, J., Lee, J.Y., Jin, G., Park, J., Huh, C.S., Kwon, I.K., Choi, Y.J. & Kong, C., 2011. Relationship between the microbiota in different sections of the gastrointestinal tract, and the body weight of broiler chickens. Springerplus. 5(1), 911.

Lan, H., Watanabe, S., Kang, D.H., Feng, L., Nakagawa, T., Kanellis, J., Mazzali, M. & Johnson, R.J., 2002. Uric acid, hominoid evolution, and the pathogenesis of salt-sensitivity. Hypertension 40(3), 355-360.

Langlois, B., Cromwell, G., Stahly, T., Dawson, K. & Hays, V., 1983. Antibiotic resistance of fecal coliforms after long-term withdrawal of therapeutic and sub-therapeutic antibiotic use in a swine herd. Appl. Environ. Microb. 46(6), 1433-1434.

Lee, C., Langlois, B.E. & Dawson, K.A., 1993. Detection of tetracycline resistance determinants in pig isolates from three herds with different histories of antimicrobial agent exposure. Appl. Environ. Microb. 59(5), 1467-1472.

Lee, K.W., Lee, S.H., Lillehoj, H.S., Li, G.X., Jang, S.I., Babu, U.S., Park, M.S., Kim, D.K., Lillehoj, E.P., Neumann, A.P. & Rehberger, T.G., 2010. Effects of direct-fed microbials on growth performance, gut morphometry, and immune characteristics in broiler chickens. Poultry. Sci 89(2), pp.203-216.

Lee, K.W., Lee, S.H., Lillehoj, H.S., Li, G.X., Jang, S.I., Babu, U.S., Park, M.S., Kim, D.K., Lillehoj, E.P., Neumann, A.P., Rehberger, T.G. & Siragusa, G.R., 2010. Effects of direct-fed microbials on growth performance, gut morphometry, and immune characteristics in broiler chickens. Poultry Sci. 89(2), 203-216.

Leeson, S. & Summers, J.D., 2001. Scotts nutrition of the chicken. 4th edn. University Books, Guelph, Ontario, Canada, pp. 394-397.

Li, X., Liu, L.Q. & Xu, C., 2008. Effects of supplementation of fructo-oligosaccharide and/or Bacillus subtilis to diets on performance and intestinal microflora in broilers. Archivf¨ur Tierzucht 51, 64-70.

Libby, D.A. & Schaible, P.J., 1955. Observations on growth responses to antibiotics and arsenic acids in poultry feeds. Science 121, 733-734.

Liljebjelke, K.A., Hofacre, C.L., White, D.G., Ayers, S., Lee, M.D. and Maurer, J.J., 2017. Diversity of antimicrobial resistance phenotypes in Salmonella isolated from commercial poultry farms. Front Vet Sci, 4, p.96.

Lin, H.S., Yue, B.D. & Ho, P.C., 2009. Determination of pterostilbene in rat plasma by a simple HPLC-UV method and its application in pre-clinical pharmacokinetic study. Biomedical Chromatography, 23(12), 1308-1315.

Linton, A., 1986. Flow of resistance genes in the environment and from animals to man. J. Antimicrob. Chemoth. 18 (Suppl. C), 189-197.

Lü, J. & Combs, Jr, G.F., 1988. Effect of excess dietary zinc on pancreatic exocrine function in the chick. J. Nutr. 118(6), 681-689.

Lü, J., Idris, U., Harmon, B., Hofacre, C., Maurer, J.J. & Lee, M.D., 2003. Diversity and succession of the intestinal bacterial community of the maturing broiler chicken. Appl. Environ. Microbiol. 69, 6816-6824.

Luo, X.G., Ji, F., Lin, Y.X., Steward, F.A., Lü, L., Liu, B. & Yu, S.X., 2005. Effects of dietary supplementation with copper sulfate or tribasic copper chloride on broiler performance, relative copper bioavailability, and oxidation stability of vitamin E in feed. Poultry Sci. 84(6), 888-893.

Ma, Y. & Guo, T., 2008. Intestinal morphology, brush border and digesta enzyme activities of broilers fed on a diet containing Cu2+-loaded montmorillonite. Brit. Poultry Sci. 49(1), 65-73.

Mackie, R.I., Sghir, A. & Gaskins, H.R., 1999. Developmental microbial ecology of the neonatal gastrointestinal tract. Am. J. Clin. Nutr. 69, 1035S.

Maenz, D.D., Engele-Schaan, C.M., Newkirk, R.W. & Classen, H.L., 1999. The effect of minerals and mineral chelators on the formation of phytase-resistant and phytase-susceptible forms of phytic acid in solution and in a slurry of canola meal. Anim. Feed. Sci. Tech. 81(3-4), 177-192.

Mahagna, M., Nir, I., Larbier, M. & Nitsan, Z., 1995. Effect of age and exogenous amylase and protease on development of the digestive tract, pancreatic enzyme activities and digestibility of nutrients in young meat-type chicks. Reprod. Nutr. Dev. 35(2), 201-212.

Marshall, B.M. & Levy, S.B., 2011. Food animals and antimicrobials: impacts on human health. Clin Microbiol Rev, 24(4), pp.718-733.

Martel, J.L. & Coudert, M., 1993. Bacterial resistance monitoring in animals, The French national experiences of surveillance schemes. Vet. Microbiol. 35(3-4), 321-338.

Martorell, R., 2002. Benefits of zinc supplementation for child growth. Am. J. Clin. Nutr. 75(6), 957-958.

McGinnis, J., Fry, R.E., Allred, J.B., & Jensen, L.S. 1958. Influence of enzyme supplementation and water treatment on the nutritional value of different grains for poults. Poultry Sci. 37(2), 372-375.

Mead, G.C. & Adams B.W., 1975. Some observations on the caecal microflora of the chick during the first two weeks of life. Brit. Poultry Sci. 16, 169-176.

Miles, R., O'keefe, S., Henry, P., Ammerman, C. & Luo, X., 1998. The effect of dietary supplementation with copper sulfate or tribasic copper chloride on broiler performance, relative copper bioavailability, and dietary prooxidant activity. Poultry Sci. 77(3), 416-425.

Moore, P., Evenson, A., Luckey, T., McCoy, E., Elvehjem, C. & Hart, E., 1946. Use of sulfasuxidine, streptothricin, and streptomycin in nutritional studies with the chick. J. Biol. Chem. 165, 437-441.

Nandi, S., Maurer, J.J., Hofacre, C. & Summers, A.O., 2004. Gram-positive bacteria are a major reservoir of Class 1 antibiotic resistance integrons in poultry litter. Proceedings of the National Academy of Sciences of the United States of America, 101(18), 7118-7122.

National Research Council (NRC). 1980. Guide for the care and use of laboratory animals. Nat. Acad. Press, Washington, DC.

National Research Council (NRC). 1994. Nutrient requirements of poultry. 9th ed. Nat. Acad. Press, Washington, DC.

Ndi, O. and Barton, M., 2012. Antibiotic resistance in animals–the Australian perspective. Antimicrobial Resistance in the Environment, pp.265-290.

Nesse, L.L., Bakke, A.M., Eggen, T., Hoel, K., Kaldhusdal, M., Ringø, E. & Yazdankhah, S.P., 2015. The risk of development of antimicrobial resistance with the use of coccidiostats in poultry diets. Opinion of the Panel on Animal Feed of the Norwegian Scientific Committee for Food Safety.

Niewold, T., 2007. The nonantibiotic anti-inflammatory effect of antimicrobial growth promoters, the real mode of action? A hypothesis. Poultry Sci. 86(4), 605-609.

Nurmi, E. & Rantata, M., 1973. New aspects of Salmonella infection in broiler production. Nature 241, 210–211.

Nys, Y., Gautron, J., McKee, M.D., Garcia-Ruiz, J.M. & Hincke, M.T., 2001. Biochemical and functional characterisation of eggshell matrix proteins in hens. World Poultry Sci. J. 57(4), 401-413.

Oluwasile, B.B., Agbaje, M., Ojo, O.E. and Dipeolu, M.A., 2014. Antibiotic usage pattern in selected poultry farms in Ogun state. Sokoto J. Vet Sci, 12(1), pp.45-50.

Ondracek, K.M., Applegate, T.J., Pang, Y.F., Thompson, K.L. & Jaynes, P., 2002. The effects of copper sulfate on the efficacy of phytase, growth, and phosphorus retention in broiler chicks. Poultry Sci. 81(Suppl. 1), 11.

Paloheimo, M., Piironen J. & Vehmaahperä J., 2010. Xylanases and cellulases as feed additives. In: Enzymes in farm animal nutrition. Eds: Bedford, M.R. & Partridge, G.G., CABI, Cambridge, England, pp. 12-53.

Pang, Y., Patterson, J. & Applegate, T., 2009. The influence of copper concentration and source on ileal microbiota. Poultry Sci. 88(3), 586-592.

Patterson, J.A. & Burkholder, K.M., 2003. Application of prebiotics and probiotics in poultry production. Poultry Sci. 82, 627-631.

Persia, M., Dehority, B. & Lilburn, M., 1999. Cecal bacterial concentrations in turkey hens fed corn or wheat-based diets with and without supplemental enzymes. Poultry Sci. 78(Suppl. 1), 16.

Pesti, G.M. & Bakalli, R.I., 1996. Studies on the feeding of cupric sulfate pentahydrate and cupric citrate to broiler chickens. Poultry Sci. 75(9), 1086-1091.

Phillips, I., Casewell, M., Cox, T., De Groot, B., Friis, C., Jones, R., Nightingale, C., Preston, R. & Waddell, J., 2004. Does the use of antibiotics in food animals pose a risk to human health? A critical review of published data. J. Antimicrob. Chemother. 53, 28–52.

Podbielski, A., Boeckh, C. & Haller, B., 2000. Growth inhibitory activity of gutta-percha points containing root canal medications on common endodontic bacterial pathogens as determined by an optimized quantitative in vitro assay. J. Endodont. 26(7), 398-403.

Quesada, A., Ugarte-Ruiz, M., Iglesias, M.R., Porrero, M.C., Martínez, R., Florez-Cuadrado, D., Campos, M.J., García, M., Píriz, S., Sáez, J.L. & Domínguez, L., 2016. Detection of plasmid mediated colistin resistance (MCR-1) in Escherichia coli and Salmonella enterica isolated from poultry and swine in Spain. Res Vet Sci, 105, pp.134-135.

Ratcliff, J., 2003. Antibiotic bans—a European perspective. Proceedings of the 47th Maryland Nutrition Conference for Feed Manufacturers, Maryland, pp. 135-152.

Rath, N.C., Balog, J.M., Huff, W.E., Huff, G.R., Kulkarni, G.B. & Tierce, J.F., 1999. Comparative differences in the composition and biomechanical properties of tibiae of seven-and seventy-two-week-old male and female broiler breeder chickens. Poultry Sci. 78(8), 232-1239.

Reid, C-A. & Hillman K., 1991. The effects of retrogradation and amylose/amylopectin ratio of starches on carbohydrate fermentation and microbial populations in the porcine colon. Anim. Sci. 68(3), 503-510.

Reti, K.L., Thomas, M.C., Yanke, L.J., Selinger, L.B. and Inglis, G.D., 2013. Effect of antimicrobial growth promoter administration on the intestinal microbiota of beef cattle. Gut pathogens, 5(1), p.8.

Richards, J.D., Zhao, J., Harrell, R.J., Atwell, C.A. & Dibner, J.J., 2010. Trace mineral nutrition in poultry and swine. Asian-Australas. J. Anim. Sci. 23(11), 1527-1534.Roselli, M., Finamore, A., Garaguso, I., Britti, M.S. & Mengheri, E., 2003. Zinc oxide protects cultured enterocytes from the damage induced by Escherichia coli. J. Nutr. 133(12), 4077-4082.

Richards, M.P. & Augustine, P.C., 1988. Serum and liver zinc, copper, and iron in chicks infected with Eimeria acervulina or Eimeria tenella. Biol. Trace Elem. Res. 17(1), 207-219.

Rollins, L.D., Gaines, S,A., Porcurull, D.W, Mercer HD & Frobish, L.T., 1976. Persistence of transferable drug resistance in the lactose-fermenting enteric ¯ora of swine following antimicrobial feeding. Canadian Journal of Comparative Medicine 40, 175 – 183.

Rosen, G.D., 1996. The nutritional effects of tetracyclines in broiler feeds. In: XX World's Poultry Congress pp. 141-146.

Rucker, R.B., Kosonen T., Clegg, M.S., Mitchell, A.E., Rucker, B.R., Uriu-Hare, J.Y. & Keen C.L., 1998. Copper, lysyl oxidase, and extracellular matrix protein cross-linking. Am. J. Clin. Nutr. 67, 996S-1002S.

SA, A.F., El-Sanhoury, M.H., El-Mednay, N.M. and Abdel-Azeem, F., 2008. Thyroid activity, some blood constituents, organs morphology and performance of broiler chicks fed supplemental organic acids. Int J. Poultry Sci, 7(3), pp.215-222.

Sahin, K., Sahin, N., Kucuk, O., Hayirli, A. & Prasad, A., 2009. Role of dietary zinc in heat-stressed poultry, A review. Poultry Sci. 88(10), 2176-2183.

Salim, H.M., Kang, H.K., Akter, N., Kim, D.W., Kim, J.H., Kim, M.J., Na, J.C., Jong, H.B., Choi, H.C., Suh, O.S. and Kim, W.K., 2013. Supplementation of direct-fed microbials as an alternative to antibiotic on growth performance, immune response, cecal microbial population, and ileal morphology of broiler chickens. Poultry Sci. 92(8), pp.2084-2090.

Salminen, S., Bouley, C., Boutron, M.C., Cummings, J.H., Franck, A., Gibson, G.R., Isolauri, E., Moreau, M.C., Roberfroid, M. & Rowland, I., 1998. Functional food science and gastrointestinal physiology and function. Brit. J. Nutr. 80(S1), S147-S171.

Salminen, S., Von Wright, A., Morelli, L., Marteau, P., Brassart, D., De Vos, W.M., Fondén, R., Saxelin, M., Collins, K., Mogensen, G. & Birkeland, S.E., 1998. Demonstration of safety of probiotics – a review. Int. J. Food Microbiol. 44(1-2), 93-106.

Savage, D.C., 1977. Microbial ecology of the gastrointestinal tract. Annu. Rev. Microbiol. 31, 107-133.

Savage, D.C., 1989. Gastrointestinal microflora in mammalian nutrition. Annu. Rev. Nutr. 6, 155-178.

Sawai, J., 2003. Quantitative evaluation of antibacterial activities of metallic oxide powders (ZnO, MgO and CaO) by conductimetric assay. J. Microbiol. Meth. 54(2), 177-182.

Schneitz, C., 2005. Competitive exclusion in poultry – 30 years of research. Food control, 16(8), 657-667.

Shankar, A.H. & Prasad, A.S., 1998. Zinc and immune function: the biological basis of altered resistance to infection. Am. J. Clin. Nutr. 68(2), 447S-463S.

Simons, P., Versteegh, H.A., Jongbloed, A.W., Kemme, P.A., Slump, P., Bos, K.D., Wolters, M.G., Beudeker, R.F. & Verschoor, G.J., 1990. Improvement of phosphorus availability by microbial phytase in broilers and pigs. Brit. J. Nutr. 64(2), 525-540.

Simpson, J.M., McCracken, V.J., Gaskin, H.R. & Mackie R.I., 1999. Application of denaturant gradient gel electrophoresis for the analysis of the porcine gastrointestinal microbiota. J. Microbiol. Meth. 36, 167-179.

Skinner, J.T., Izat, A.L. & Waldroup, P.W., 1991. Research note, Fumaric acid enhances performance of broiler chickens. Poultry Sci. 70(6), 1444-1447.

Smith, H.W., 1967. The effect of the use of antibacterial drugs, particularly as food additives, on the emergence of drug-resistant strains of bacteria in animals. New Zeal. Vet. J. 15(9), 153-166.

Smith, H.W., 1973. Effect of prohibition of the use of tetracyclines in animal feeds on tetracycline resistance of faecal E.coli of pigs. Nature. 243, 237-238.

Snel, J., Harmssen, H.J.M., Van der Wielen, P.W.J.J. & Williams, B.A., 2002. Dietary strategies to influence the gastrointestinal microflora of young animals, and its potential to improve intestinal health. In: Nutrition and Health of the Gastrointestinal Tract. Eds: Blok M.C., Vahl, H.A., De Lange, L., Van de Braak, A.E., Hemke, G. & Hessing, M., Wageningen Academic Publishers, The Netherlands, pp. 37-69.

Söderberg, T.A., Gref, R., Holm, S., Elmros, T. & Hallmans, G., 1990. Antibacterial activity of rosin and resin acids in vitro. Scand. J. Plast. Reconstr. Hand. Surg. 24, 199-205.

Stanton, T.B., 2013. Butyrate-producing bacteria, including mucin degraders, from the swine intestinal tract. Appl. Environ. Microb. 79(12), 3879-3881.

Starr, M.P. & Reynolds, D.M., 1951. Streptomycin resistance of coliform bacteria from turkeys fed streptomycin. American Journal of Public Health and the Nations Health, 41, 1375-1380.

Stutz, M. & Lawton, G.C., 1984. Effects of diet and antimicrobials on growth, feed efficiency, intestinal Clostridium perfringens, and ileal weight of broiler chicks. Poult Sci. 63(10), 2036-2042.

Sutter, V.L. & Finegold, S.M., 1976. Susceptibility of anaerobic bacteria to 23 antimicrobial agents. Antimicrob Ag Chemother. 10(4), 736-752.

Suwalsky, M., Ungerer, B., Quevedo, L., Aguilar, F. & Sotomayor, C., 1998. Cu 2+ ions interact with cell membranes. J. Inorg. Biochem. 70(3), 233-238.

Tabata, E., Kashimura, A., Wakita, S., Ohno, M., Sakaguchi, M., Sugahara, Y., Kino, Y., Matoska, V., Bauer, P.O. and Oyama, F., 2017. Gastric and intestinal proteases resistance of chicken acidic chitinase nominates chitin-containing organisms for alternative whole edible diets for poultry. Scientific reports, 7(1), p.6662.

Underwood, J. & Suttle, F., 1999. The detection and correction of mineral imbalances. Mineral nutrition of livestock, 3.

Upadhayay, U.P.P.D.D. & Vishwa, P.C.V., 2014. Growth Promoters and Novel Feed Additives Improving Poultry Production and Health, Bioactive Principles and Beneficial Applications: The Trends and Advances—A Review. Int J. Pharmacol, 10(3), pp.129-159.

Valfre, F., 1983. Nature and physiology of performance promoters. Zootec. Int. 7, 40–46.

Van den Bogaard, A.E. & Stobberingh, E.E., 2002. Antibiotic resistance of faecal enterococci in poultry, poultry farmers and poultry slaughterers. J. Antimicrob. Chemoth. 49(3), 497-505.

van den Bogaard, A.E. and Stobberingh, E.E., 2000. Epidemiology of resistance to antibiotics: links between animals and humans. Int. J Antimicrob Ag 14(4), pp.327-335.

Van der Wielen, P.W., Keuzenkamp, D.A., Lipman, L.J., Van Knapen, F. & Biesterveld, S., 2002. Spatial and temporal variation of the intestinal bacterial community in commercially raised broiler chickens during growth. Microb. Ecol. 44, 286-293.

Vieira, S.L., 2007. Chicken embryo utilization of egg micronutrients. Braz J. Poult Sci, 9(1), pp.1-8.

Visek, W.J., 1978. The mode of growth promotion by antibiotics. J. Anim. Sci. 46, 1447-1469.

Viveros, A., Brenes, A., Pizarro, M. & Castano, M., 1994. Effect of enzyme supplementation of a diet based on barley, and autoclave treatment, on apparent digestibility, growth performance and gut morphology of broilers. Anim. Feed Sci. Tech. 48(3), 237-251.

Viveros, A., Chamorro, S., Pizarro, M., Arija, I., Centeno, C. and Brenes, A., 2011. Effects of dietary polyphenol-rich grape products on intestinal microflora and gut morphology in broiler chicks. Poult, Sci 90(3), pp.566-578.

Vogt, H., Matthes, S. & Harnisch, S., 1981. The effect of organic acids in the rations on the performances of broilers and laying hens (in German). Archiv fur Geflugelkunde. 45, 221-231.

Vos, E., 2000. Reforming the European Commission: What role to play for EU agencies. Common Market L. Rev., 37, pp.1113.

Waibel, P.E., Abbott, O.J., Baumann, C.A. & Bird, H.R., 1954. Disappearance of the growth response of chicks to dietary antibiotics in an "old" environment. Poult Sci. 33(6), 1141-1146.

Wang, Z., Qiao, S., Lü, W. & Li, D., 2005. Effects of enzyme supplementation on performance, nutrient digestibility, gastrointestinal morphology, and volatile fatty acid profiles in the hindgut of broilers fed wheat-based diets. Poultry Sci. 84(6), 875-881.

Wiley, E.O. and Lieberman, B.S., 2011. Phylogenetics: theory and practice of phylogenetic systematics. John Wiley & Sons.

Wise, M. & Siragusa, G., 2007. Quantitative analysis of the intestinal bacterial community in one- to three-week-old commercially reared broiler chickens fed conventional or antibiotic-free vegetable-based diets. J. Appl. Microbiol. 102(4), 1138-1149.

Wong, L., 2001. The market in Chinese social policy (pp. 38-62). Basingstoke: Palgrave.

Wray, C., Hedges, R., Shannon, K. & Bradley, D., 1986. Apramycin and gentamicin resistance in Escherichia coli and salmonellas isolated from farm animals. J. Hyg-Cambridge 97(3), 445-456.

Xia, M., Hu, C. & Xu, Z., 2004. Effects of copper-bearing montmorillonite on growth performance, digestive enzyme activities, and intestinal microflora and morphology of male broilers. Poultry Sci. 83(11), 1868-1875.

Yadav, A., Rai, M., Ingle, A.P., Birla, S. & Santos, C.A.D., 2016. Strategic role of selected noble metal nanoparticles in medicine. Crit. Rev. Microbiol. 42(5), 696-719.

Zanella, I., N.K. Sakomura and F.G. Silversides, 1999. Effect of supplementation of broiler diets based on corn and soybeans. Poult. Sci. 78, 561-568.

Zhang, B., Shao, Y., Liu, D., Yin, P., Guo, Y. & Yuan, J., 2012. Zinc prevents Salmonella enterica serovar Typhimurium – induced loss of intestinal mucosal barrier function in broiler chickens. Avian Pathology 41(4), 361-367.

Zimbro, M.J., Power, D.A. (Eds.), 2003. Difco & BBL Manual. Bekton, Dickinson and Co., Sparks, MD, pp. 121–124.

3 CHAPTER THREE -EFFECTS OF ZOOTECHNICAL FEED ADDITIVES ON GROWTH PERFORMANCE, BONE LINEAR MEASUREMENTS AND BLOOD PARAMETERS IN BROILER CHICKENS

Abstract

This study was designed to investigate the effects of zootechnical feed additives [chelated minerals (Zn, Cu and Mn), a live Bacillus probiotic ($\geq 4 \times 10^9$ CFU per gram, protease enzyme (600 000 U/g, EC 3.4.21.62), and fumaric and benzoic organic acids] on growth parameters, bone measurements and blood parameters of broiler chickens. Eight hundred Cobb broiler chickens were evenly allocated to 40 pens (experimental units) to which five dietary treatments were randomly assigned: a negative control (NC) consisting of a commercial broiler diet without Zn-bacitracin (ZnB) antibiotic growth promoter; a positive control consisting of a commercial broiler diet with Zn-bacitracin antibiotic growth promoter (PCZnB); NC + protease enzyme + chelated minerals (MinEnz); NC + protease enzyme + chelated minerals + Organic acids (MinEnz+Org); NC + protease enzyme + chelated minerals + probiotic (MinEnzProb),. Feed intake (FI), weight gain and feed conversion efficiency were determined weekly. Blood was collected from chickens on the 33rd day and 2 days before slaughter to determine haematological and serum biochemical parameters. On day 35 chickens were slaughtered for assessment of bone development parameters. There were no significant ($P>0.05$) dietary effects on body weight gain (BWG) of chickens throughout the feeding trial save for the first week. The chickens fed MinEnz showed the highest body weight (17.36 ± 0.45 g) in the first week while chickens fed PCZnB showed the lowest weight (15.25 ± 0.45 g). Experimental diets had an effect on the FI in the first three weeks of the trial with broilers fed MinzEnz+Pro consuming the most feed (20.87 ± 0.53 g) in the first week. In the

second week both MinEnz and MinEnz+Org diets promoted the highest FI (58.18 ± 1.01 g) while the PCZnB-fed chickens had the least intake (54.31 ± 1.01 g). The negative control (NC) chickens had the highest FI in the third week (119.55 ± 3.13 g) while MinEnz chickens had the lowest. In the last two weeks, no dietary influence (P>0.05) was observed on FI. Diet had no effect on the full blood indices except for haemoglobing and haemocrit (HTC). The chickens fed MinEnzPro (9.57 ± 0.32 g/dl) and NC (9.52 ± 32 g/dl) had the highest haemoglobin levels. The NC promoted the highest HTC value compared to all the other dietary treatments. Among serum biochemical parameters, only alanine aminotransferase (ALT) and total serum protein were affected by the diet. The PCZnB chickens had the highest ALT level (8.50 ± 1.57 IU/L) while NC birds had the highest level of total serum protein (33.37 ± 1,14 g/l). They were no dietary effects observed in all bone development parameters. It was concluded that the proposed zootechnical feed additives combinations can be used as alternatives to Zn-Bacitracin antibiotic since they produce similar results in growth parameters, bone linear measurements and blood parameters.

Keywords: Zootechnical feed additives, Growth performance, Bone linear measurements & Broiler chickens

3.1 Introduction

The gastrointestinal tract (GI tract) helps with digestion and absorption of nutrients that are required for growth and maintenance. The multiplication of pathogenic microbes in the GI tract results in high mortalities, decrease productivity and contaminate poultry products. Antibiotics have been used sub-therapeutically over the years in broiler diets to avoid necrotic infections of the gut by controlling pathogenic bacteria. There have been concerns regarding the use of in-feed antibiotics due to the development of antibiotic-resistant bacteria. These public concerns have resulted in a decrease in the use of in-feed antibiotics in food animals (Smith et al., 2003). Consequently, there is a rising demand for alternatives to in-feed antibiotics that will improve gut integrity, performance and safety of broiler products. Examples of these alternatives include: organic acids, enzymes, probiotics and chelated minerals. Most of these proposed alternatives have a direct or indirect effect on gut microflora (Richards, 2005).

Organic acids are well known for their strong bacteriostatic effects on the gut microflora and they have been used over the years for the control of salmonella (Ricke, 2003). Short chain organic acids (formic, fumaric and propionic), have been reported to minimise colonisation of pathogenic bacteria and production of toxic metabolites (Kirchgessner & Roth, 1988). Probiotics have a number of health benefits beyond providing basic nutritional advantages. Probiotic products consisting of beneficial microflora can help to establish and maintain the balance of the intestinal microflora in commercial broilers (Netherwood et al., 1999; Fritts et al., 2000). The main mechanism of action of probiotics is through the establishment of a normal gut microflora by minimising or decreasing the disturbances caused by enteric pathogens (Dhama et al., 2008). The

effectiveness of the probiotic depends on the type and concentration of the probiotic and physiological state of the bird. Probiotic strains should be able to resist acid, bile salts and digestive enzymes and, most importantly, must be able to reproduce rapidly. The strain must also not pose any danger or should not impart any antibiotic resistance into the intestinal microflora (Seema & Johri, 1992; Pal & Chander, 1999; Dhama et al., 2011; Mookiah et al., 2014).

Exogenous enzymes are potential alternatives to antibiotic growth promoters (AGPs) because of their ability to improve digestibility of a wide range of feed components such as protein, phytate and fibre. The effects of exogenous enzymes to the gut microflora occur in the ileal and caecal phases (Bedford, 2000). In the ileal phase, enzymes decrease the number of pathogenic microbes by speeding up the digestion process and limiting the number of substrate available. Trace minerals such as manganese (Mn), zinc (Zn) and copper (Cu) are necessary for various body functions and are also vital to maintaining optimum health (Dieck et al., 2003). Both exogenous and endogenous enzymes require the presence of minerals for their activity. It is important to use the required optimal levels of trace minerals to allow the animals to reach their full genetic potential performance (Leeson & Caston, 2007). Copper plays an important role as a component of many enzymes which have an effect on the maturation of haematopoetic cells; copper deficiency can lead to inadequate iron utilisation in animals (Aksu et al., 2010). The trace mineral zinc plays a vital role in the process of erythropoiesis. It particularly helps as a catalyst in the activity of α-aminolevunilic acid dehydratase, which plays a part in haem thesis. Deficiency of zinc can lead to adverse effects on erythropoeisis in marrow and a decrease of T and B lymphocyte production.

Several studies have indicated the positive effects of probiotics (Kalavathy et al., 2008; Awad et al., 2009), organic acids (Abdel-Fattah et al., 2008) and chelated minerals (Yan & Waldroup, 2006; Yi et al., 2007) on broiler performance and health, but no studies have investigated the possible

additive effects of these additives when used in combination. Indeed, when used separately, the outcomes have been inconsistent across studies (Ferket, 2004; Patterson & Burkholder, 2003; Diarra & Malouin, 2014.

Therefore, the objective of this study was to evaluate the effects of a live Bacillus probiotic ($\geq 4 \times 10^9$ CFU per gram), a protease enzyme (600 000 U/g; EC no: 3.4.21.62), chelated minerals (Zn, Cu and Mn), and fumaric and benzoic acids as feed additives, in place of AGP ZnB, on FI, growth performance, bone development and blood parameters of broiler chickens. Thus, the study tested the hypothesis that zootechnical feed additives improve the growth performance, feed utilisation efficiency and blood parameters of broiler chickens.

3.2 Material and methods

3.2.1 Study site

The research trial was conducted at the North-West University Experimental Farm (Molelwane) in the North West province of South Africa. The geographical coordinates are 25.8560°S, 25.6403°E. The farm is located at an altitude of 1782 above sea level. The annual rainfall is between 300 and 500 mm, while the temperature ranges from 3°C - 37°C.

3.2.2 Feed ingredients

All the zootechnical feed additives were supplied by Novus International. The protease enzyme used had an activity of 600 000 U/gram. The probiotic consists of a dried Bacillus licheniformis PWD-1 that is not less than 4×10^9 CFU fermentation soluble mixed with limestone and natural flavour. The minerals (copper, zinc and manganese) used were chelated with methionine hydroxyl analogue butanoic acid (HMTBa). The organic acid that was used in this study was a combination

of benzoic acid and fumaric acids blended to HMTBa. The inclusion levels of chelated minerals, protease enzyme, organic acids and probiotic were 0.03, 0.05, 0.5 and 0.02%, respectively. The chelated minerals additive was a composite of 19.3% copper, 36% zinc, and 44.7% manganese by weight.

3.2.3 Chicks, management and experimental design

Eight hundred day-old Cobb chicks were purchased from a local hatchery (Mimosa Chicks, Lichtenburg) and were weighed before being randomly and evenly distributed into 40 pens (20 chicks/pen). The pens, measuring (1.5m x 1.3m x 2m), were designed to meet animal welfare standards for optimal production. Ethical clearance was sought and obtained from the Animal Research Ethics Committee of the North-West University. Five dietary treatments were each randomly allocated to eight replicate pens (experimental units) in a completely randomised design. The dietary treatments under investigation were as follows:

1. A negative control consisting of a commercial broiler diet without Zn-bacitracin antibiotic growth promoter (NC)

2. A positive control consisting of a commercial broiler diet with Zn-bacitracin antibiotic growth promoter (PCZnB)

3. NC + protease enzyme + chelated minerals (MinEnz)

4. NC + protease enzyme + chelated minerals + fumaric and benzoic organic acids (MinEnzOrg)

5. NC + protease enzyme + chelated minerals + live Bacillus probiotic (MinEnzProb).

The chelated minerals feed additive consisted of zinc, copper and manganese.

Table 3.1. Ingredient composition (%) of the dietary treatments at starter, grower and finisher phase

	Dietary treatments[1]														
	Starter					Grower					Finisher				
Ingredients	T1	T2	T3	T4	T5	T1	T2	T3	T4	T5	T1	T2	T3	T4	T5
Corn fine ground	43.0	43.0	43.0	43.0	43.0	46.23	46.23	46.23	46.23	46.23	51.47	51.47	51.47	51.47	51.47
Soy oilcake ARG	16.0	16.0	16.0	16.0	16.0	16.0	16.0	16.0	16.0	16.0	13.0	13.0	13.0	13.0	13.0
Sunflower oilcake	5.0	5.0	5.0	5.0	5.0	0	0	0	0	0	0	0	0	0	0
Wheat bran SA	32.5	32.5	32.5	32.5	32.5	34.5	34.5	34.5	34.5	34.5	32.5	32.5	32.5	32.5	32.5
Limestone	1.1	1.1	1.1	1.1	1.1	1.0	1.0	1.0	1.0	1.0	0.95	0.95	0.95	0.95	0.95
MCP	1.1	1.1	1.1	1.1	1.1	0.95	0.95	0.95	0.95	0.95	0.8	0.8	0.8	0.8	0.8
Salt	0.15	0.15	0.15	0.15	0.15	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Synth lysine	0.39	0.39	0.39	0.39	0.39	0.28	0.28	0.28	0.28	0.28	0.26	0.26	0.26	0.26	0.26
MHA	0.29	0.29	0.29	0.29	0.29	0.24	0.24	0.24	0.24	0.24	0.22	0.22	0.22	0.22	0.22
Synth threonine	0.15	0.15	0.15	0.15	0.15	0.1	0.15	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Axtra PHY dry	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Broiler starter premix	0.25	0.25	0.25	0.25	0.25	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.2	0.2	0.2
ZN BAC 15%	0	0.05	0	0	0	0	0.05	0	0	0	0	0.05	0	0	0
Monensin 20%	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Sodium BIC	0.3	0.3	0.3	0.3	0.3	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Organic acids	0	0	0	0.50	0	0	0	0	0.50	0	0	0	0	0.5	0
Probiotic	0	0	0	0	0.02	0	0	0	0	0.02	0	0	0	0	0.02
Chelated minerals	0	0	0.03	0.03	0.03	0	0	0.03	0.03	0.03	0	0	0.03	0.03	0.03
Protease	0	0	0.05	0.05	0.05	0	0	0.05	0.05	0.05	0	0	0.05	0.05	0.05

[1]Dietary treatments: T1 = a negative control consisting of a commercial broiler diet without Zn-bacitracin antibiotic growth promoter; T2 = a positive control consisting of a commercial broiler diet with Zn-bacitracin antibiotic growth promoter; T3 = T1 + protease + chelated minerals; T4 = T1 + protease + chelated minerals + fumaric and benzoic organic acids; T5 = T1 + protease + chelated minerals + live Bacillus probiotic; ARG = Argentina; SA= South Africa; MCP= Mono-calcium phosphate; Synth= Synthesis; MHA= 2-hydro-4-methylthio butanoic acid; PHY= Phytase; ZN BAC= Zinc Bacitracin.

Table 2.2 Chemical composition (%, unless otherwise stated) of stater, grower and finisher diets for broilers

Ingredients	Starter	Grower	Finisher
Metabolisable energy (MJ/Kg)	10.1	9.8	9.5
Dry matter	87.7	87.6	87.6
Organic matter	82.0	82.2	82.6
Crude protein	18.2	16.8	15.4
Ether extract	3.26	3.36	3.42
Crude fibre	6.11	5.36	5.14
Calcium	0.81	0.73	0.66
Sodium	0.14	0.14	0.14
Chlorine	0.25	0.25	0.25
Digestible LYS	0.76	0.85	0.98
Digestible MET	0.41	0.44	0.51
Digestible M+S	0.63	0.67	0.78
Digestible THR	0.54	0.58	0.68
Digestible TRP	0.12	0.14	0.15
Digestible ARG	0.75	0.83	0.91
Digestible ILE	0.50	0.55	0.60

3.2.4 Feeding management

The chickens received a low density diet (15% below the minimum requirements stated in the Cobb 500 manual) in Table 3. At the end of the starter phase on day 13, the birds were fasted for

16 hours. On days 14 to 16, the birds received a high protein diet, consisting of 40% soybean meal and 10% poultry by-products, in order to induce stress as described by Kocher et al. (2004). The grower diet in Table 3 was then introduced on day 17. The birds were also stressed using a high stocking density. The recommended stocking density for broilers is 35 kg/m^2 (16 birds) while for this trial it was 42 kg/m^2. The feeders were placed at the corners of the pens to decrease feeder space. All these challenges were designed to upset the gut and stress the birds to see if the tested zoonotic feed additives will have any effect under these harsh rearing conditions.

Day	Event
1	Starter (Crumbs)
2	
11	
12	
13	• Start 16 hour fast
14	• Change of feed: from crumbs to mash • High protein feed
15	• Change of feed: from crumbs to mash • High protein feed
16	• Change of feed: from mash to pellets • High protein feed
17	• End of the challenge • Pellet feeding till day 35
18	
19	
20	

3.2.5 Feed utilisation and growth performance

Live body weight and FI were measured weekly. Body weight gain (BWG) was calculated on a weekly basis throughout the 35-day experimental period. Cumulative feed intake was calculated

at the end of the experiment. Feed conversion ratio (FCR) was calculated by the following equation:

$$\text{FCR} = \frac{Cumulative\ feed\text{int}\,ake\,(g/bird)}{Weight\ gain\,(g/bird)}$$

Mortality was recorded daily, and mortality rate was calculated at the end of the experiment.

3.2.6 Blood sampling and analysis

Two broiler chickens from each pen were randomly selected at day 33 for blood collection. Blood samples were collected in non-heparised (red tube) and EDTA (purple tube) tubes by puncturing the brachial vein. Within two hours of collection, blood was transported to Lancet laboratory (Mafikeng, SA). The purple tube with anti-coagulant was used for haematological analysis. The Idexx lasercyte (Haematology analyser) was used to analyse for haematocrit (HTC), haemoglobin, erythrocyte, leucocytes, neutrophils, lymphocytes, monocytes, eosinophils, and normoblasts. The red tube blood was centrifuged to obtain serum. The total protein, albumin, aspartate aminotransferase (AST), alanine transaminase (ALT), sodium, potassium, albumin, urea, calcium and cholesterol were quantified using an auto-analyser (Hitachi-704, Boehringer Mannheim Ldt, Germany).

3.2.7 Latency-to-lie, tibia linear parameters and bone breaking strength

At day 35, two broiler chickens per treatment were exposed to the latency-to-lie test, as described by Berg and Sanotra (2003). This test is based on the fact that body interaction with water is a novel experience for broiler chickens. Each bird was individually placed in a plastic container previously filled with 3 cm of water at 32 °C. The time it takes for the bird to sit down was

recorded, according to the principle that the better leg health the broilers has, the longer it will stand up to avoid body contact with the water. If the chicken remained standing after 10 minutes, the test was interrupted, because the legs were deemed to be strong and healthy.

At day 35 of the trial, eight right tibiae were collected from each treatment (one tibia per replicate pen) through surgical removal during necropsy. Tibiae muscles were gently removed and cleaned, including removal of periosteum by hand. Tibia length diameter, tibia diameter proximal end and tibia diameter distal were measured using a Toshiba-Rotanode x-ray (Toshiba Electro Tubes and Devices, Tochigi, Japan) connected to the Point-of-Care (CR140) system for digital imaging. After linear measurements, all the tibia bones were placed on an adjustable 3-point bend/snap fixture fitted on a heavy-duty TA-XT platform of a texture analyser (model TA-XT plus, Stable Microsystems, Surrey, UK) and broken with a 6 cm flat head probe attached to a 50 kg load cell reporting the breaking force in Newtons. The distance to the bone support was 50 mm. The breaking bone strength was recorded as the peak load prior to the bone breakage.

3.2.8 Statistical analysis

Overall FI, BWG, and FCR, blood parameters, latency-to-lie and tibia linear data were analysed using GLM procedures of SAS (2010) according to the following statistical model:

$$Y_{ij} = \mu + T_i + E_{ij}$$

Where: Y_{ij} = dependent variable, μ = population mean constant common to all observations, T_i = effect of diet, and E_{ij} = random error term. For all tests, the level of significance was set at $P<0.05$.

$Yijk = \mu + Ti + Wj + (T * W)ij + \varepsilon ijk$ (2)

Where: Yij = observation (Cumulative weight gain parameter), μ = population mean constant common to the observation, Ti = effect of diet, T*Wij = effect of diet interacting with week

and εij = random error term. For all tests, the level of significance was set at (P <0.05).

3.3 Results

3.3.1 Feed intake and growth performance

Repeated measures analysis showed that there was no week × diet interaction effect on FI, BWG and FCR, therefore, overall performance data is presented. FI and growth performance results are shown in Table 3.1. At day 35, there were no dietary effect (P>0.05) on FI. The antibiotic growth promoter diet (PCZnB) (positive control) promoted similar (P>0.05) BWG as the other four dietary treatments. The addition of probiotic (MinEnzProb) and organic acids (MinEnzOrg) to diet containing chelated minerals and protease enzyme had no effect (P>0.05) on BWG. Similarly, FCR was not influenced (P>0.05) by dietary treatments.

Table 3.3: The effect of zootechnical feed additives on overall feed intake (g), weight gain (g) and feed conversion ratio of broilers

	Dietary treatments[1]					
	NC	PCZnB	MinEnz	MinEnzOrg	MinEnzProb	SEM
Weight gain	2682	2823	2767	2807	2807	83.00
Feed intake	3299	3165	3275	3310	3313	71.38
Feed conversion ratio	1.23	1.14	1.18	1.17	1.18	0.03

[1]Dietary treatments: NC = a negative control consisting of a commercial broiler diet without Zn-bacitracin antibiotic growth promoter; PCZnB = a positive control consisting of a commercial broiler diet with Zn-bacitracin antibiotic growth promoter; MinEnz = NC + protease enzyme +

chelated minerals; MinEnzOrg = NC + protease enzyme + chelated minerals + fumaric and benzoic organic acids; MinEnzProb = NC + protease enzyme + chelated minerals + live Bacillus probiotic.

3.3.2 Haematological and serum biochemistry

The effects of zootechnical feed additives on haematological parameters are represented in Table 3.2. As indicated in the table, dietary treatments did not have any significant effect ($P>0.05$) on erythrocytes, leucocytes, neutrophils, lymphocytes, monocytes, eosinophils, basophils and normaplasts. Dietary effect ($P<0.05$) was only observed on haemoglobin and HTC. Chickens fed MinEnzProb (9.57 ± 0.32 g/dl) and NC (9.52 ± 0.32 g/dl) had the highest haemoglobin levels. The addition of probiotic improved haemoglobin levels compared to the diet with PCZnB, which promoted the least haemoglobin levels compared to all dietary treatments. The NC diet promoted the highest HTC value (0.37 ± 0.01%) compared to all other treatments. The chickens on PCZnB diet had the least HTC values when compared to chickens fed diets with zootechnical feed additives.

Table 3.4: The effect of zootechnical feed additives on haematology of broiler chickens

Parameters[2]	Dietary treatments[1]					SEM
	NC	PCZnB	MinEnz	MinEnzOrg	MinEnzPro	
Erythrocytes (x 10^{12}/l)	2.68	2.39	2.59	2.63	2.68	0.10
Haemoglobin (g/dl)	9.52^{bc}	8.33^{a}	9.37^{b}	9.37^{b}	9.57^{c}	0.32
Haematocrit (g/dl)	0.37^{c}	0.31^{a}	0.34^{b}	0.34^{b}	0.36^{bc}	0.01
Leucocytes (x 10^{9}/l)	26.50	22.69	27.27	22.06	23.33	4.06
Neutrophils (x 10^{9}/l)	1.18	0.89	0.62	0.91	0.52	0.38
Lymphocytes (x 10^{9}/l)	15.03	16.82	22.50	18.53	19.49	2.85
Monocytes (x 10^{9}/l)	0.66	1.27	0.73	0.95	0.79	0.38
Eosinophils (x 10^{9}/l)	1.85	1.31	1.36	1.34	1.57	0.60
Basophils (x 10^{9}/l)	0.84	0.50	2.04	0.11	0.94	0.36
Normoplasts (/100WBC)	3160	2946	2553	3030	3103	287

[1]Dietary treatments: NC = a negative control consisting of a commercial broiler diet without Zn-bacitracin antibiotic growth promoter; PCZnB = a positive control consisting of a commercial broiler diet with Zn-bacitracin antibiotic growth promoter; MinEnz = NC + protease enzyme + chelated minerals; MinEnzOrg = NC + protease enzyme + chelated minerals + fumaric and benzoic organic acids; MinEnzProb = NC + protease enzyme + chelated minerals + live Bacillus probiotic

[2]Parameters: HTC = Haematocrit; ALT = alanine aminotransferase AST = Aspartate aminotransferase.

[abc] Means between treatments groups without a common superscript are significantly different ($P<0.05$).

The blood biochemistry indices are also represented in Table 3.3. There was a lack of statistical significance ($P>0.05$) of dietary treatments with respect to serum aspartate aminotransferase (AST), potassium, albumin, urea, calcium corrected and cholesterol levels. The alanine aminotransferase (ALT) content of the blood was affected ($P<0.05$) by the diet with chickens fed PCZnB having the highest (8.50 ± 1.57 IU/L) level, followed by NC chickens (5.75 ± 1.57 IU/L). Chickens fed both controls (NC and PCZnB) had higher ALT than those fed diets with zootechnical feed additives. The addition of organic acids to the diet with chelated minerals and protease enzyme (MinEnzOrg) resulted in higher ALT levels in chickens. Addition of organic acids to the diet with chelated minerals and protease enzyme (MinEnzOrg) also resulted in higher serum ALT levels compared to the addition of probiotics (MinEnzPro). Total serum protein was significantly ($P<0.05$) influenced by diet, with the highest level (33.37 ± 1.14 g/l) being observed in NC chickens and the lowest level in PCZnB chickens (29.75 ± 1.14 g/l). Both MinEnzPro and MinEnzOrg diets promoted higher serum protein level compared to the chickens receiving PCZnB or MinEnz diets.

Sodium content was also affected ($P<0.05$) by the diet with chickens fed MinEnz having the least concentration (144.0 ± 1.25 mmol/l) while the NC chickens had the highest concentration (147.25 ± 1.25 mmol/l). The inclusion of zinc-bacitracin antibiotic (ZnB) and zootechnical feed additives did not affect sodium levels when compared to the negative control diet. With regards to urea levels in serum chickens fed NC diet had the highest urea levels compared to all dietary groups.

Table 3.5. The effect of zootechnical feed additives on serum biochemistry of broiler chickens

Parameters[2]	Dietary treatments[1]					SEM
	NC	PCZnB	MinEnz	MinEnzOrg	MinEnzPro	
ALT (IU/L)	5.75^{b}	8.50^{c}	2.25^{a}	3.37^{ab}	2.87^{a}	1.57
AST (IU/L)	391.25	336.87	380.62	358.50	265.75	36.26
Total protein (g/l)	33.37^{c}	29.75^{a}	29.50^{a}	31.75^{b}	31.00^{b}	1.14
Sodium	147.25^{c}	144.50^{b}	144.00^{a}	144.25^{b}	144.25^{b}	1.25
Potassium (mmol/l)	5.98	6.03	5.38	6.23	5.82	0.38
Albumin (g/l)	13.75	12.80	12.62	13.75	13.87	0.44
Urea (mmol/l)	0.39^{c}	0.33^{b}	0.20^{a}	0.37^{bc}	0.31^{b}	0.05
Calcium total (mmol/l)	2.66	2.57	2.71	2.81	2.71	0.12
Calcium corrected (mmol/l)	3.19	3.12	3.27	3.09	3.24	0.06
Cholesterol (mmol/l)	3.73	3.61	3.46	3.72	3.72	0.14

[1]Dietary treatments: NC = a negative control consisting of a commercial broiler diet without Zn-bacitracin antibiotic growth promoter; PCZnB = a positive control consisting of a commercial broiler diet with Zn-bacitracin antibiotic growth promoter; MinEnz = NC + protease enzyme + chelated minerals; MinEnzOrg = NC + protease enzyme + chelated minerals + fumaric and benzoic organic acids; MinEnzProb = NC + protease enzyme + chelated minerals + live Bacillus probiotic

[2]Parameters: HTC = Haematocrit; ALT = alanine aminotransferase AST = Aspartate aminotransferase.

[abc]Means between treatments groups without a common superscript are significantly different ($P<0.05$).

The effects of zootechnical feed additives on bone development are presented in Table 3.3. Diets did not have any influence (P>0.05) on all the bone development parameters. The addition of zootechnical feed additives produced similar results as both controls (NC and PCZnB). Similarly, no statistical differences (P>0.05) were observed between the zootechnical feed additive-diets (MinEnz, MinEnzOrg and MinEnzPro).

Table 3.6: The effect of zootechnical feed additives on bone development parameters

	Dietary treatments[1]					
Parameters	NC	PCZnB	MinEnz	MinEnzOrg	MinEnzPro	SEM
Latency-to-lie (seconds)	246.37	350.25	209.87	302.37	411.50	87.60
Tibia length (cm)	8.92	9.24	9.27	9.00	8.99	0.12
Tibia proximal (cm)	2.03	2.05	2.02	2.17	2.16	0.07
Tibia distal (cm)	1.66	1.86	1.75	1.80	1.76	0.05
Bone breaking strength (kg)	22.86	22.64	21.12	23.51	28.03	2.37

[1]Dietary treatments: NC = a negative control consisting of a commercial broiler diet without Zn-bacitracin antibiotic growth promoter; PCZnB = a positive control consisting of a commercial broiler diet with Zn-bacitracin antibiotic growth promoter; MinEnz = NC + protease enzyme + chelated minerals; MinEnzOrg = NC + protease enzyme + chelated minerals + fumaric and benzoic organic acids; MinEnzProb = NC + protease enzyme + chelated minerals + live Bacillus probiotic

3.4 Discussion

3.4.1 Growth performance

Feed intake (FI), BWG and FCR measurements were not affected by the dietary treatments at the end of the experiment. Similar findings were reported by Cave (1984); Watkins and Kratzer (1984); Izat et al. (1990); Lee et al. (1993); Engberg et al. (2000); Panda et al. (2000); Ceylan et al. (2003); Öztürk and Yildirin (2004); and Vale et al. (2004), who found that dietary inclusion of AGPs, probiotics and organic acids had no effect on both BWG and FCR. There are also reports (Gunal et al., 2006; Nollet et al., 2007) indicating that high levels of dietary organic acid have a negative impact on performance parameters of broilers. Indeed, Cave (1984) discovered that increased levels of propionic acid decreased FI and BWG in broilers. In contrast, several other researchers reported beneficial effects of these additives on both the BWG and FCR (Vogt et al., 1981; Henry et al., 1987; Manickam et al., 1994; Runho et al., 1997; Yeo & Kim, 1997; Gunes et al., 2001). In general, most reported results in the available literature on the effects of zootechnical feed additives on broiler performance are inconsistent. A number of factors including the environment, general management, nutrition, additive types, dosage and bird characteristics (age, species, stage of production) can affect broiler responses to feed additives (Yang et al., 2009) and thus could explain these contrasting results.

Angel et al. (2005) reported that when broilers are grown under favourable conditions, in the absence of stress and diseases, the inclusion of dietary probiotics does not result in any beneficial effects on growth parameters. Chicks that are well-nourished and reared under optimal conditions (clean environment and at moderate stocking density) do not respond positively to growth promoters. A number of studies (Lyons, 1987; Miller, 1987; Anderson et al., 1999) reported that

when broilers are reared under clean conditions, the addition of growth promoters such as organic acids, probiotic or antibiotics do not effectively improve performance parameters. In an attempt to address this, broilers in this study were deliberately reared under moderately stressful conditions through short-term dietary manipulation and long-term higher-than-normal stocking density. The result was that the ZnB AGP had no distinct advantage over the preferred zootechnical feed additives in terms of broiler growth performance.

In the present study the proposed zootechnical feed additives produced similar results with the ZnB control diet, which suggest that probiotics and organic acids have same beneficiary effects as antibiotics growth promoters. Similar results were reported by Alp et al. (1999), Kahraman et al. (1999), and Patterson and Burkholder (2003).

3.4.2 Haematological parameters

Nutritional factors (diet quantity, form and composition) also affect intermediary metabolism, resulting in changes in plasma metabolite levels in poultry (Buyse et al., 2002; Swennen et al., 2005). Interestingly, this study showed that all full blood counts, apart from haemoglobin and HTC counts, were not influenced by dietary treatments. The combination of chelated minerals, protease enzyme and a probiotic (MinEnzPro) promoted the highest haemoglobin concentration in broilers compared to other treatments in the present study. This is in agreement with the study done by Cetin et al. (2005), who found that addition of a probiotic (live Bacillus spp.) to the diet caused an increase in both haemoglobin concentration and HTC values of turkeys. In contrast, Baidya et al. (1994) reported that inclusion of probiotics and antibiotics in broiler diets did not affect haemoglobin concentration. Mohan et al. (1996) reported a significant reduction in haemoglobin concentration upon dietary inclusion of a probiotic (live Bacillus spp.). The researchers suggested

that this reduction may be due to competition between the probiotic and the host for folic acid or other nutrients.

Serum biochemical parameters can be used as indicators of physiological changes happening to an organism under the influence of internal and external factors (Toghyani et al., 2010). Alanine transaminase concentration was very high in chickens on the negative control diet compared to all diets including organic acids additives. This confirms earlier findings (Hernández et al., 2006) that organic acid addition had no effect on the blood metabolites in the broiler chickens. Total protein and cholesterol levels were not affected by any of the dietary treatments in the current study. The findings are in agreement with those of Dimcho et al. (2005) who reported that probiotic inclusion did not affect the total protein concentration of broilers. The treatment with no additives (NC) had the highest level of urea and sodium compared to all the treatments.

3.4.3 Bone measurements

Skeletal problems can be caused in a variety of ways with nutrition being one of the main factor influencing skeletal development in birds. Fast growing birds have higher requirement for specific nutrients; thus skeletal defects are common in fast growing chickens (Havenstein et al., 1994) perhaps due to the inadequacy of the diet to supply that specific nutrient. As shown in Table 3.3, all bone measurements were not affected by dietary treatments. Addition of these zootechnical feed additives had no beneficial effects on bone development in contrast to Mutus et al. (2006), who showed positive effects of probiotics on tibia ash and phosphorus concentration of broilers – findings that suggest that probiotics increase both bone development and mineralisation. Probiotics have a variety of mechanisms; one of them is the synthesis of vitamins such as Vit D {those involved in bone formation????], through which they can beneficially affect bone health.

Furthermore, anti-arthritic effects of probiotics, releasing minerals from the phytic acid complex and improvement of calcium absorption by enterocytes are other proposed modes of action for positive effects of probiotics on bone health and development (Scholz-Ahrens et al., 2007). In the present study, the dietary addition of organic acid did not affect any bone measurements. The results are in contrast with Brenes et al. (2003) who reported that supplementation with citric acid to broiler diets improves utilisation of minerals (Ca, P and Zn). The anion of an acid can complex with minerals such as Ca, P, Mg and Zn, subsequently improving the mineral's digestibility (Edwards and Baker, 1999).

3.5 Conclusions

Results showed that the inclusion of a combination of zootechnical feed additives in broilers diets produce similar growth performance, blood parameters and bone development indices in the same way as the diet containing the AGP ZnB. The zootechnical feed additive did not promote undesirable results in terms of performance parameters, blood parameters and bone development indices of the broiler chickens. Haematological indices were observed to be within the range for healthy broilers for all the treatments. Supplementation with these zootechnical feed additives can be considered a viable alternative to AGPs since they produce similar effects on growth parameters, blood parameters and bone measurements.

3.6 References

Abdel-Fattah, S. A., EI-Sanhoury, M. H., EI-Mednay, N. M., & F. Abdul-Azeem., 2008. Thyroid activity of broiler chicks fed supplemental organic acids. Int. J. Poult. Sci. 7, 215–222.

Alp, M., Kocabağli, N., Kahraman, R. & Bostan, K., 1991. Effects of dietary supplementation with organic acids and zinc bacitracin on ileal microflora, pH and performance in broilers. Turk. J. Vet. Anim. Sci. 23(5), 451-456.

Anderson, D.B., McCracken, J.J., Mamirov, R.I., Simpson, J.M., Mackie, R.I., Vestegen, H.R. & Gaskins, H.R., 1999. Gut microbiology and growth promoting antibiotics in swine. Pig News & Info. 20, 115N-122N.

Angel, R., Dalloul, R.A. & Doerr, J., 2005. Performance of broiler chickens fed diets supplemented with a direct-fed microbial. Poultry Sci. 84, 1222-1231.

Award, W.A., Ghareeb, K., Abdel-Raheem, S., & Böhm, J. 2009. Effect of dietary inclusion of probiotic and synbiotic on growth performance, organ weights, and intestinal histomorphology of broiler chickens. Poultry Sci. 88 (1), 49–56.

Baidya, N., Mandal, L., Sarkar, S. K. & Banerjee, G. C. 1994. Combined feeding of antibiotic and probiotic on the performance of broilers. Indian J. Poult. Sci, 29, 228-231.

Bedford, M., 2000. Removal of antibiotic growth promoters from poultry diets, implications and strategies to minimise subsequent problems. World Poultry Sci. J. 56, 347-365.

Bedford, M.R. & Cowieson, A.J., 2012. Exogenous enzymes and their effects on intestinal microbiology. Anim. Feed Sci. Tech. 173, 76-85.

Brenes, A., Viveros, A., Arija, I., Centeno, C., Pizarro, M. & Bravo, C., 2003. The effect of citric acid and microbial phytase on mineral utilization in broiler chicks. Anim Feed Sci & Tech. 110, 201-219.

Buyse, J., Jassens, K., Van Der Geyten, S., Van As, P., Decuypere, E. & Darras, V.M., 2002. Pre- and postprandial changes in plasma hormone and metabolite levels and hepatic deiodinase activities in meal-fed broiler chickens. Br. J. Nutr. 88, 641e53.

Cave, M.M., 1984. Effects of dietary propionic acid on feed intake in chicks. Poultry Sci., 63, 131-134.

Cetin, N., Güçlü, B.K., & Cetin, E., 2005. The effects of probiotic and mannanoligosaccharide on some haematological and immunological parameters in Turkeys. J. Vet. Med. A. Physiol. Pathol. Clin. Med. 52, 263-267.

Ceylan, N., Ciftci, I., Ildiz, F. & Sogut, A., 2003. Etlik pilic rasyonlarina enzim, buyutme faktoru, probiyotic ve organic asit ilavesinin besi performansi ve bagirsak mikroflorasina etkileri. A,U. Tarim Bilimleri Dergisi. 9, 320-326

Dhama, K., Mahendran, M., Tomar, S. & Chauhan, R.S., 2008. Beneficial effects of probiotics and prebiotics in livestock and poultry, the current perspectives. Intas. Polivet. 9, 1-13.

Dhama, K., Verma V., Samant, P.M., Tiwari, R., Vaid, R.K. & Chauhan, R.S., 2011. Application of probiotics in poultry, Enhancing immunity and beneficial effects on production performances and health. A review. J. Immuno. Immunopatho. 13, 1-19.

Dibner, J.J. & Buttin, P., 2002. Use of organic acids as a model to study the impact of gut microflora on nutrition and metabolism. J. Appl. Poultry Res. 11(4), 453-463.

Dieck, H.T., Doring, F., Roth, H.P. & Daniel, H., 2003. Changes in rat hepatic gene expression in response to zinc deficiency as assessed by DNA arrays. J. Nutr. 133, 1004-1010.

Dimcho, D., Svetlana, B., Tsvetomira, S., & Tatiana, V., 2005. Effect of feeding Lactina probiotic on performance, some blood parameters and caecal microflora of mule ducklings. 2005. Trakia J. Sci. 3 (2): 22-28.

Djouvinov, D., Boicheva, S., Simeonova, T. & Vlaikova, T., 2005. Effect of feeding lactina probiotic on performance, some blood parameters and caecal microflora of mule ducklings. Trakia J. Sci. 3(2), 22-28.

Edwards, H.M. & Baker, D.H., 1999. Bioavailability of zinc in several sources of zinc oxide, zinc sulfate, and zinc metal. J Anim Sci. 77(10), 2730-2735.

Engberg, R.M., Hedemann, M.S., Leser, T.D. & Jensen, B.B., 2000. Effect of zinc bacitracin and salinomycin on intestinal microflora and performance of broilers. Poultry Sci. 79, 1311-1319.

Fritts, C. A., Kersey, J. H., Motl, M. A., Kroger, E. C., Yan, F., Si, J., Jiang, Q., Campos, M. M., Waldroup, A. L. & Waldroup P. W., 2000. Bacillus subtilis C-3102 (Calsporin) improves live performance and microbiological status of broiler chickens. J. Appl. Poult. Res. 9:149–155.

Gunal, M., Yayli, G., Kaya, O., Karahan, N. and Sulak, O., 2006. The effects of antibiotic growth promoter, probiotic or organic acid supplementation on performance, intestinal microflora and tissue of broilers. Int. J. Poult. Sci, 5(2), pp.149-155.

Gunes, H., Cerit, H. & Altinel, A., 2001. Etlik piliçlerin verim özellikleri üzerine pre-probiotigin (Fermacto-500) etkisi. Ist. Univ. Vet. Fak. Derg. 27, 217-229.

Havenstein, G.B., Ferket, P.R., Scheideler, S.E. & Larson, B.T., 1994. Growth, livability, and feed conversion of 1957 vs 1991 broilers when fed “typical” 1957 and 1991 broiler diets. Poultry Sci. 73, 1785-1794.

Henry, P.R., Ammerman, C.B., Campbell, D.R. & Miles, R.D., 1987. Effect of antibiotics on tissue trace mineral concentration and intestinal tract weight of broiler chicks. Poultry Sci. 66(6), 1014-1018.

Hernández, F., Garcia, V., Madrid, J., Orengo, J., Catala, P. & Megias, M.D., 2006. Effect of formic acid on performance, digestibility, intestinal histomorphology and plasma metabolite levels of broiler chickens. Brit. Poultry Sci. 47(1), 50-56.

Izat, A.L., Tidwell, N.M., Thomas, R.A., Reiber, M.A., Adams, M.H., Colberg, M. & Waldroup, P.W., 1990. Effects of a buffered propionic acid in diets on the performance of broiler chickens and on microflora of the intestine and carcass. Poultry Sci. 69(5), 818-826.

Kahraman, R., Abas, I., Bostan, K., Tanör, M.A., Kocabağli, N. & Alp, M., 1999. Effects of organic acids and yeast culture on performance ileum pH and Enterobacteriaceae population of broilers. VIP Poultry Yutav’99 Istanbul S, 515-522.

Kalavathy, R., Abdullah, N., Jalaludin, S., Wong, C.M.V.L. and Ho, Y.W., 2008. Effect of Lactobacillus cultures and oxytetracycline on the growth performance and serum lipids of chickens. Int. J. Poultry Sci. 7(4), 385-389.

Kiarie, E., Romero, L.F. & Nyachoti, C.M., 2013. The role of added feed enzymes in promoting gut health in swine and poultry. Nutr. Res. Rev. 26, 71-88.

Kirchgessner, V.M. & Roth, F.X., 1988. Ergotrope effekte durch organische sauren in der ferkelaufzucht und schweinemast. Ubers. Tierernaehr, 16, 93-108.

Kocher, A., Rodgers, N.J. & Choct, M., 2004. Efficacy of alternatives to IFAs in broilers challenged with Clostridium perfringens. In: Proceedings of the Australian Poultry Science Symposium 16:130-133.

Lee, S.J., Kim, S.S., Suh, O.S., Na, J.C., Lee, S.H. & Chung, S.B., 1993. Effect of dietary antibiotics and probiotics on the performance of broiler. RDA J Agric Sci (Korea Republic).

Leeson, S. & Summers, J.D., 2001. Scott's nutrition of the chicken. 4th edn. University Books, Guelph, Ontario, Canada, pp. 341-363.

Leeson, S., & Caston, L., 2008. Using minimal supplements of trace minerals as a method of reducing trace mineral content of poultry manure. Anim. Feed Sci. Technol. 142, 339-347.

Lyons, T.P., 1987. Probiotics an alternative to antibiotics. Pig News Info. 8, 157-164.

Manickam, R., Viswanathan, K. & Mohan, M., 1994. Effects of probiotics in broiler performance. Ind. Vet. J. 71, 737-739

Miller, B.F., 1987. Acidified poultry diets and their implications for poultry industry. In, Biotechnology in the feed Industry. Alltech Technical Publications, Kentuky, pp. 199-209.

Mohan, B., Kadirvel. R., Natarajan A., & Bhaskaran, M., 1996. Effect of probiotic supplementation on growth, nitrogen utilisation and serum cholesterol in broilers. Br. Poult. Sci. 37, 395–401.

Mookiah, S.S., Sieo, C.C., Ramasamy, K., Abdullah, N. & Ho, Y.W., 2014. Effects of dietary prebiotics, probiotic and symbiotic on performance, caecal bacterial populations and caecal fermentation concentrations of broiler chickens. J. Sci. Food Agric. 94, 341-348.

Mutus, R., Kocabag, N., Alp, M., Acar, N., Eren, M. & Gezen, S.S., 2006. The effect of dietary probiotic supplementation on tibial bone characteristics and strength in broilers. Poultry Sci. 85, 1621-1625.

Netherwood, T., Gilbert, H.J., Parker, D.S. & O'donnell, A.G., 1999. Probiotics shown to change bacterial community structure in the avian gastrointestinal tract. Appl Environ Microbio. 65(11), 5134-5138.

Nollet, L., Van der Klis, J.D., Lensing, M. and Spring, P., 2007. The effect of replacing inorganic with organic trace minerals in broiler diets on productive performance and mineral excretion. J. Appl Poult Res, 16(4), pp.592-597.

Öztürk, E. & Yildirim., 2004. Probiyotiklerin etlik piliclerin perfomancy ve bagirsak mikrobiyolojik ozelliklerine elkileri 4. Ulusal Zootekni Bilin Kongresi. 1-3 eylül. Ciltz. Poster Bildiriler, S, 297-303

Pal, P. & Chander, U., 1999. Probiotics benefits. Poultry Int. October issue, pp. 40-44.

Panda, A.K., Reddy, M.R., Rama Rao, S.V., Raju, M.V. & Praharaj, N.K., 2000. Growth, carcass characteristics, immune competence and response to Escherichia coli of Broiler fed diets with various levels of probiotic. Archive fur Geflugelkunde, 64, 152-156.

Patterson, J.A. & Burkholder, K.M., 2003. Application of prebiotics and probiotics in poultry production. Poultry Sci. 82, 627-631.

Rebolé, A., Ortiz, L.T., Rodríguez, M.L., Alzeuta, C., Treviño, J. & Velasco, S., 2010. Effects of inulin and enzyme complex, individually or in combination, on growth performance, intestinal microflora, cecal fermentation characteristics, and jejunal histomorphology in broiler chickens fed a wheat- and barley-based diet. Poultry Sci. 89, 276-286.

Richards, J.D., Gong, J. & De Lange, C.F.M., 2005. The gastrointestinal microbiota and its role in monogastric nutrition and health with an emphasis on pigs, Current understanding, possible modulations and new technologies for ecological studies. Can. J. Anim. Sci. 85, 421-435.

Ricke, S.C., 2003. Perspectives on the use of organic acids and short chain fatty acids as antimicrobials. Poultry Sci. 82, 632-639.

Runho, R.C., Sakomura, N.K., Kuana, S., Banzatto, O., Junoqueria, M. & Stringhini, J.H., 1997. Uso do acido organoco (acido fumarico) nas racoes de frangos de corte. Revista Brasileira de Zootecnia. 26, 1183-1191.

Scholz-Ahrens, K., Ade, P., Marten, B., Weber, P., Timm, W., Asil, Y., Gluer, C.C. & Schrezenmeir, J., 2007. Prebiotics, probiotics, and symbiotics affect mineral absorption, bone mineral content, and bone structure. J. Nutr. 137, 838-846.

Seema, A. & Johri, T.S., 1992. Probiotics in poultry feeds. Poultry Guide. 29, 51-58.

Smith, D.L., Johnson, J.A., Harris, A.D., Furuno, J.P., Perencevich, E.N. & Morris J.G., 2003. Assessing risks for a pre-emergent pathogen, Virginiamycin use and the emergence of streptogramin resistance in Enterococcus faecium. Lancet Infect. Dis. 3, 241-249.

Swennen, Q., Janssens, G.P.J., Millet, S., Vansant, G., Decuypere, E. & Buyse, J., 2005. Effects of substitution between fat and protein on feed intake and its regulatory mechanisms in broiler chickens, Endocrine functioning and intermediary metabolism. Poultry Sci. 84(7), 1051-1057.

Timmerman, H.M., Veldman, A., Van den Elsen, E., Rombouts, F.M. & Beynen, A.C., 2006. Mortality and growth performance of broilers given drinking water supplemented with chicken-specific probiotics. Poultry Sci. 85, 1383-1388.

Toghyani, M., Tohidi, M., Gheisari, A.A. & Tabeidian, S.A., 2010. Performance, immunity, serum biochemical and hematological parameters in broiler chicks fed dietary thyme as alternative for an antibiotic growth promoter. Afri J Biotech. 9(40), 6819-6825.

Vale, M.M., Menten, J.M.F., Morais, S.C.D. & Brainer, M.M.A., 2004. Mixture of formic acid and propionic acid as additives in broiler feeds. Scintia Agricola Piracicaba 61, 371-375.

Verstegen, M.W.A. & Williams, B.A., 2002. Alternatives to the use of antibiotics as growth promoters for monogastric animals. Anim. Biotech. 13, 113-127.

Vogt, H., Matthes, S. & Harnisch, S., 1981. The effect of organic acids in the rations on the performances of broilers and laying hens (in German). Archiv fur Geflugelkunde. 45, 221-231.

Watkins, B.A, & Kratzer, F.H, 1984. Drinking water treatment with commercial preparation of concentrated Lactobacillus culture for broiler chickens. Poultry Sci. 63, 1671-1673.

Yan, F. & Waldroup, P.W., 2006. Evaluation of Mintrex® manganese as a source of manganese for young broilers. Int. J. Poult. Sci. 5, 708-713.

Yang, Y., Iji, P.A. & Choct, M., 2009. Dietary modulation of gut microflora in broiler chickens, a review of the role of six kinds of alternatives to in-feed antibiotics. World Poultry Sci. J. 65, 97-114.

Yeo, J. & Kim, K., 1997. Effects of feeding diets containing an antibiotic, a probiotic or yucca extract on growth and intestinal urease activity in broiler chicks. Poultry Sci. 76, 381-385.

Yi, Z., Kornegay, E.T. & Denbow, D.M. (2007) Supplemental microbial phytase improves zinc utilization in broilers. Poultry Sci. 75, 540–546.

4 CHAPTER FOUR: EFFECTS OF ZOOTECHNICAL FEED ADDITIVES ON CARCASS CHARACTERISTICS AND MEAT QUALITY OF BROILER CHICKENS

Abstract

This study was to designed to investigate the effects of zootechnical feed additives (chelated minerals (Zn, Cu and Mn), a live Bacillus probiotic, a protease enzyme and fumaric and benzoic organic acids) on carcass characteristics and meat quality of Cobb broiler chickens. Eight hundred Cobb broiler chickens were allocated to 40 pens (experimental units), to which were randomly assigned five dietary treatments: a negative control consisting of a commercial broiler diet without Zn-bacitracin antibiotic growth promoter (NC); a positive control consisting of a commercial broiler diet with Zn-bacitracin antibiotic growth promoter (PCZnB); NC + protease enzyme + chelated minerals (MinEnz); NC + protease enzyme + chelated minerals + organic acids (MinEnzOrg); NC + protease enzyme + chelated minerals + probiotic (MinEnzProb),. The chickens were fed and reared for 35 days as described in Chapter 3 above. At day 35, chickens were slaughtered for assessment of carcass traits and meat quality parameters. The weights of the heart, full gizzard, empty gizzard and the length of the small intestine were not influenced ($P>0.05$) by dietary treatments. However, diets influenced ($P<0.05$) the weights of spleen and proventriculus, with the heaviest spleens (3.81 ± 0.32 g) and proventriculi (12.63 ± 0.61 g) measured in chickens fed MinPro. No significant ($P>0.05$) dietary effect was observed on breast, drumstick, wing, head, and shank weights, but on neck weights. Negative control chickens had the heaviest (55.50 ± 2.02 g) necks while MinPro chickens had the lightest (44.88 ± 2.08 g) neck weights. Initial pH, drip loss, shear force, lightness (L*) and yellowness (b*) were not ($P>0.05$) influenced by diet. However, redness (a*) was significantly ($P<0.05$) affected by diet, with meat

from the positive control chickens having the highest (3.17 ± 0.67 g) value. Similarly, meat from the same diet also had the highest water holding capacity (WHC) (15.92 ± 1.34 %) and cooking loss (26.03 ± 1.35 %). Results showed that using zootechnical feed additives as alternatives to antibiotic growth promoters (AGPs) can be considered since the additives did not have a negative impact on carcass characteristics and meat quality parameters compared to the positive control of antibiotics.

Keywords: Zootechnical feed additive, meat quality, carcass traits, and broiler chickens

4.1 Introduction

The success of poultry production enterprises is directly dependent on meat quality and carcass yield. Inherent characteristics of the bird (Northcutt et al., 2003; Lyon et al., 2004) and pre-slaughter conditions (e.g. feeding level, heating, housing system, ventilation and lighting, pre-harvest time off feed, capture and loading) (Lyon et al., 2004; Karacay et al., 2008) are contributing factors to carcass yield and meat quality (Fletcher, 2002; Delezie et al., 2007). Poultry breeders have put intense focus on growth parameters (growth velocity and muscle mass) as the major breeding objectives in the last 30 years. The growth parameters continue to influence the decision-making in poultry production due to the need to minimise costs, but are being questioned and criticised after the appearance of morphological disorders in skeletal muscles (Sosnicki & Wilson, 1991). The criteria used for poultry carcass grading are mainly based on bruising and discolouration, which remains biased since it doesn't take into account other important qualities to the processor and the consumer. The current shift in the market from whole birds to further processed products has challenges related with toughness and poor cohesiveness, WHC problems and colour (Sosnicki & Wilson, 1991).

Appearance is the major criterion for purchase selection and initial evaluation of meat quality by customers. Additional quality parameters such as cooking loss, tenderness, juiciness, pH, dripping loss and shelf-life are vital to the consumer after purchasing as well as to the processor when producing value-added meat products (Allen et al., 1998).

Nutrition plays a major role in poultry production and its effect on meat quality has been observed over the years. Zootechnical feed additives have several advantages over commonly used

commercial antibiotics on meat quality since they are residue free and they are also, generally recognised as safe and commonly used items in the food industry (Varel, 2002).

Tenderness has been recorded as a vital sensory factor preferred by consumers (Deathrage, 1963; Savell et al., 1989, Cavitt et al., 2005). There are a number of methods used to measure tenderness including sensory, consumer analysis, instruments and descriptive (Cavitt et al., 2004). pH is another common determinant of meat quality (Battula et al., 2008). Protein denaturing may result from a rapid pH decline, which will result in a decline in tenderness and juiciness as well as pale colour of the muscle (Solomon et al., 1998). Meat colour is a significant attribute for the consumer's initial selection, final determination of acceptability post cooking and prior to consumption (Fletcher, 1999). The WHC of meat is directly influenced by pH and denaturing of proteins (Offer & Knight, 1988; Van Laack et al., 2000).

Minimal research has been reported on the effects of combinations of zootechnical feed additives such as organic acids, probiotics, chelated trace minerals and enzymes on broiler carcass and meat quality traits. Therefore, this study was done to evaluate the effects of the combination of these zootechnical feed additives on broiler carcass and meat quality traits, which was evaluated through tenderness, colour, pH, cooking loss, dripping loss and organ weights. The objective of this experiment was to test whether the suggested zootechnical feed additives will have better effects than antibiotics growth promoters on both meat quality and carcass traits.

4.2 Materials and methods

4.2.1 Study site, source of diets, animal management

The full description of the study area is given in section 3.2.1 while section 3.2.2 provides a full description of the experimental design and dietary treatments.

4.2.2 Slaughter procedure

At day 35, all the chickens were deprived feed for 13 hours to allow for the emptying of the crop (Ari et al., 2013). The broilers were taken to the nearest local abattoir for slaughter (Rooigrond Braaikuikens abattoir A3/32) situated in Mafikeng, South Africa. Upon arrival at the abattoir, the broilers, grouped by pen (experimental unit), were immediately put into a metal rack upside down for stunning. They were then slaughtered by the cutting of the jugular vein with a sharp knife and allowing bleeding to take place for 2 minutes. Chickens were put inside the de-feathering machine. After removal of feathers, hot carcasses (without neck, giblets and feet) were weighed. Thereafter, all the carcasses and internal organs were taken to the university laboratory for determination of internal organ sizes. The carcasses were kept at 5°C in a cold room for 24 hours, after which the cold carcass weight was measured.

4.2.3 Carcass traits and internal organs

Carcasses from the different replicate pens were identified by placing them in marked plastic bags immediately after slaughter. Carcass evisceration and measurement of carcass traits were done at the abattoir. The following internal organs were hand-stripped and weighed: gizzard, spleen, breast, provetriculars, heart, liver and small intestines (jejunum, duodenum and ileum). The external organs that were weighed are wings, drumstick, shank, neck and breast. The carcass

weight of each chicken was recorded and dressing out percentage was calculated as: Carcass Weight / Live Weight x 100.

4.2.4 Meat colour and pH

Immediately after slaughter, meat pH was measured on the breast muscle using a Corning Model 4 pH (Corning Glass Works, Medfield, MA). Colour of the meat (L* = Lightness, a* = Redness and b*=Yellowness) was measured using a Minolta colour-guide (Spectrophotometer CM 2500c, Konika, Osaka, Japan), with a 20 mm diameter measurements area with innovative 45°a: 0 geometry optics. The colour meter was calibrated before measurements using a white tile for standard white colour calibration.

4.2.5 Water holding capacity of meat

The WHC was determined on the surface of a freshly cut slice of the pectoralis major muscle (16 - 41 grams), and was determined as the amount of water released from fresh meat held under pressure (60 kg pressure) using the filter paper press method described by Grau & Hamm, (1957). The water from the freshly cut meat was taken up by a pre-weight filter paper and calculated as a percentage. WHC was calculated as follows:

$$\text{WHC (\%)} = \frac{(\text{initial weight} - \text{weight after pressing})}{\text{initial weight}} \times 100$$

4.2.6 Meat drip loss

Drip loss was determined according to the method described by Zhang et al. (2005). The weighed pieces of muscles (w1) from the PPM were hooked and suspended using a wire in a plastic bottle and sealed properly so that the sample was not in contact with the sides of the bottle. The bottles

were refrigerated at 4°C for three days (72 hours). The meat samples were weighed again after three days to obtain the weight after drip (w2). The difference in weight of each sample before and after drip was conveyed as percentage drip loss and calculated using the following equation:

$$\text{Drip loss (\%)} = \frac{(W1 - W2)}{W1} \times 100$$

Where; w1 is initial weight, and w2 is weight after drip.

4.2.7 Meat cooking loss

The breast sample was chilled overnight at 4°C. Thereafter, the samples were individually weighed to obtain initial weight (w1) of the pectoralis major muscle after thawing. The samples were then placed in foil plates, which were placed in an oven (dry-heating) at 180°C for 30 minutes. The broiled muscles were then removed from the oven and were allowed to cool down for 20 minutes.

The samples were then re-weighed to obtain the cooked weight (w2). The cooking loss was then calculated based on the difference between the weight of raw meat and cooked meat using the following equation:

$$\text{Cooking loss (\%)} = \frac{(W1 - W2)}{W1} \times 100$$

Where: w1 is weight of raw meat, w2 is weight after cooking.

4.2.8 Meat tenderness

The cooked breast muscle that was used in the determination of cooking loss at 180°C for 30 min was also used for shear force evaluation. The cooked samples were sheared perpendicular to the fibre direction using a Meullenet – Owens Razor Shear Blade (A/MORS) mounted on a texture

analyser (TA-XT plus, Stable Micro Systems, Surry, UK). The reported value in Newtons (N) represented the average of the peak force measurements for each sample.

4.2.9 Statistical analysis

Carcass characteristics and meat quality traits data were analysed using GLM procedure of SAS (2010) according to the following statistical model:

$$Y_{ij} = \mu + T_i + \varepsilon_{ij}$$

Where: Y_{ij} = observation (carcass characteristics, meat quality parameters), μ = population mean constant common to all observations, T_i = effect of diet, and E_{ij} = random error term. For all statistical tests, the level of significance was set at $P < 0.05$.

4.3 Results

4.3.1 Carcass traits

The effects of experimental diets on carcass characteristics of broilers are presented in Table 4.1. No differences existed ($P > 0.05$) on all the carcass traits.

Table 4.1. The effects of dietary treatments on carcass characteristics of broilers

	Dietary treatments[1]					
	NC	PCZnB	MinEnz	MinEnzOrg	MinEnzProb	SEM
Final Body weight (g)	2726.2	2804.2	2773.1	2850.7	2804.3	84.6
Hot carcass (g)	1716.5	1570.5	1590.2	1673.1	1619.6	55.13
Cold carcass (g)	1684.5	1548.5	1560.6	1620.6	1631.3	53.13
Dressing %	63.95	57.22	56.50	61.67	62.34	3.05

[1]Dietary treatments: NC = a negative control consisting of a commercial broiler diet without Zn-bacitracin antibiotic growth promoter; PCZnB = a positive control consisting of a commercial broiler diet with Zn-bacitracin antibiotic growth promoter; MinEnz = NC + protease enzyme + chelated minerals; MinEnzOrg = NC + protease enzyme + chelated minerals + fumaric and benzoic organic acids; MinEnzProb = NC + protease enzyme + chelated minerals + live Bacillus probiotic.

4.3.2 Internal organs

The effects of dietary treatments on sizes of internal organs of broiler chickens are presented in Table 4.2. No dietary differences were observed ($P>0.05$) in the sizes of hearts, full gizzards, empty gizzards, liver and small intestines. However, diet affected the size of the spleen and proventriculus. The heaviest spleens were observed in chickens offered MinPro (3.81 ± 0.32 g) diet followed by the chickens offered the positive control (PCZnB) with spleens weighing 2.75 ± 0.32 g on average. The NC-fed broilers had the lightest spleens. MinPro broilers had the heaviest proventriculi followed by the PCZnB. When compared to the diet with chelated minerals and

protease enzyme (MinEnz), addition of probiotic (MinEnzPro) and organic acids (MinEnzOrg) did not affect the size of the proventriculars.

Table 4.2. The effects of dietary treatments on size of internal organs of broiler chickens

Parameters	Dietary treatments[1]					SEM
	NC	PCZnB	MinPro	MinProOrg	MinProProb	
Heart (g)	13.25	11.37	12.62	12.63	12.57	0.78
Spleen (g)	2.12[a]	2.75[ab]	3.81[b]	2.67[ab]	2.32[a]	0.32
Full gizzards (g)	39.75	42.37	44.0	47.50	42.42	2.74
Empty gizzards (g)	32.88	34.12	35.25	35.13	33.86	2.18
Liver (g)	42.63	40.75	43.50	45.25	42.14	2.17
Proventriculus (g)	11.63[bc]	9.63[a]	12.63[c]	10.13[b]	10.43[b]	0.61
Small intestines (cm)	155.75	147.44	145.81	155.57	147.64	4.43

[1]Dietary treatments: NC = a negative control consisting of a commercial broiler diet without Zn-bacitracin antibiotic growth promoter; PCZnB = a positive control consisting of a commercial broiler diet with Zn-bacitracin antibiotic growth promoter; MinEnz = NC + protease enzyme + chelated minerals; MinEnzOrg = NC + protease enzyme + chelated minerals + fumaric and benzoic organic acids; MinEnzProb = NC + protease enzyme + chelated minerals + live Bacillus probiotic.
[a,b]In a row, means with common superscripts do not differ (P > 0.05).

4.3.3 External organs

The effects of zootechnical feed additives as alternatives to Zn-bacitracin antibiotic growth promoter on external organs of broiler chickens are presented in Table 4.3. No significant (P>0.05) difference was observed in terms of the size of breast, drumstick, wing, head, and shank of the broiler chickens. The only significant effect of dietary treatments was seen on the neck weights of

the broiler chickens. The chickens offered the negative control diet had the heaviest necks (55.50 ± 2.08 g) while the MinPro and PCZnB chickens had the lowest neck weights (44.88 ± 2.08 g). The MinEnzOrg chickens had higher neck weights compared to those fed on PCZnB.

Table 4.3. The effects of dietary treatments on external organs of broiler chickens

	Dietary treatments[1]					
Parameters	NC	PCZnB	MinPro	MinProOrg	MinProProb	SEM
Breast	371.75	365.62	342.75	378.00	417	26.82
Drumstick	218.00	212.00	188.87	212	227.50	11.12
Wing	94.25	86.75	88.37	98.37	100.25	6.86
Head	49.63	51.75	57.62	48.50	52.43	2.26
Shank	42.86	38.00	42.25	44.25	37.14	2.35
Neck	[b]55.50	[a]48.88	[a]44.88	[b]54.75	[ab]48.71	2.08

[1]Dietary treatments: NC = a negative control consisting of a commercial broiler diet without Zn-bacitracin antibiotic growth promoter; PCZnB = a positive control consisting of a commercial broiler diet with Zn-bacitracin antibiotic growth promoter; MinEnz = NC + protease enzyme + chelated minerals; MinEnzOrg = NC + protease enzyme + chelated minerals + fumaric and benzoic organic acids; MinEnzProb = NC + protease enzyme + chelated minerals + live Bacillus probiotic.

[a,b]In a row, means with common superscripts do not differ (P > 0.05).

4.3.4 Meat quality measurements

The effects of zootechnical feed additives as alternatives to Zn-bacitracin antibiotic growth promoter on meat quality measurements of the broilers are represented in Table 4.4. There was no significant dietary effect ($P>0.05$) on pH, dripping loss and shear force. Similarly, diets had no effect on all meat colour parameters apart from redness. Chickens fed PCZnB (3.17 ± 0.67) had the highest redness value while those fed MinPro had the lowest value. Dietary treatments influenced ($P < 0.05$) WHC with the highest value seen in chickens fed PCZnB (15.92 ± 1.34 %) and the lowest value (12.21 ± 1.34 %) in chickens fed MinProProb. For cooking loss, chickens fed NC showed the highest value (27.15 ± 1.35 %) while the chickens fed MinProOrg showed the least cooking loss (21.65 ± 1.35 %).

Table 4.4. The effects of dietary treatments on meat quality traits of broiler chickens

Parameters[2]	Dietary treatments[1]					SEM
	NC	PCZnB	MinEnz	MinEnzOrg	MinEnzProb	
Initial pH	7.01	7.16	7.11	7.10	7.11	0.05
Meat colour						
Lightness (L*)	69.83	67.24	60.04	68.27	66.95	3.16
Redness (a*)	2.17^{ab}	3.17^{b}	1.35^{a}	2.14^{ab}	2.22^{ab}	0.67
Yellowness (b*)	14.04	13.79	11.69	13.67	14.28	0.89
WHC	22.32^{c}	15.92^{b}	15.74^{b}	12.53^{a}	12.21^{a}	1.34
Drip loss (%)	87.25	85.71	36.39	88.12	80.69	2.14
Cooking loss (%)	27.15^{b}	26.03^{b}	23.79^{ab}	21.45^{a}	21.65^{a}	1.35
Shear force	10.39	8.64	9.16	9.23	9.29	1.51

[1]Dietary treatments: NC = a negative control consisting of a commercial broiler diet without Zn-bacitracin antibiotic growth promoter; PCZnB = a positive control consisting of a commercial broiler diet with Zn-bacitracin antibiotic growth promoter; MinEnz = NC + protease enzyme + chelated minerals; MinEnzOrg = NC + protease enzyme + chelated minerals + fumaric and benzoic organic acids; MinEnzProb = NC + protease enzyme + chelated minerals + live Bacillus probiotic.

[2]Parameters: WHC = Water holding capacity

[a,b]In a row, means with common superscripts do not differ (P > 0.05).

4.4 Discussion

4.4.1 Carcass traits

Final body weight, hot carcass weight, cold carcass weight, and dressing percentage were not affected by the inclusion of all the zootechnical feed additives. Moreira et al. (2001) and Vargas et al. (2002) reported similar findings when they found no differences in carcass yields between birds that were fed a probiotic (live Bacillus) and control birds. In contrast, Pelicano et al. (2003) reported that control birds showed higher carcass weights when compared to the treatments that were given probiotics (live Bacillus). Based on the present study, probiotics do not have any effects on carcass traits. The organic acids also had no effect ($P>0.05$) on the carcass traits. Skinner et al. (1991) carried-out a study comparing the effects of dietary fumaric acid on broiler performance from day 0 to day 49 and reported similar results to this study. Even the positive control diet in this study, which contained antibiotic growth promoter, had similar ($P>0.05$) carcass traits as the rest of the diets. Visek et al. (1978) demonstrated that the inclusion of antibiotics as feed additives reduces the weight and the length of the intestines in poultry, which has a direct effect on carcass yield. The lack of positive effect of PCZnB diet might be attributed to this observation.

The lack of uniformity in the effects of zootechnical feed additives on carcass traits across studies could be explained by Emmans' theory, which postulates that animals have "inherent" carcass composition based on their genetic makeup, but vary in response to environment and nutritional factors (Emmans, 1981). Thus, different studies testing the same additives may produce inconsistent results due to environmental variation.

4.4.2 Internal organs

In the present study, there were no dietary effects on size of internal organs apart from the spleen and the proventriculus (Table 4.2). The lack of dietary influence on the size of internal organs has also been reported in other studies (Cai et al., 2015; Zhang et al., 2013). The reason for this outcome is largely unknown, but could be due to random effect (Cai et al., 2015). In this study, the weight of the spleen was higher in broilers fed the protease enzyme and chelated minerals. and similar results have been reported by Award et al. (2009). The measurements of immune organ weights can be used as a method to evaluate the immune status in chickens (Heckert et al., 2002).

4.4.3 External organs

Dietary inclusion of organic acids in this study did not affect the size of external organs measured except for neck weights. No comparisons were made with other studies because there are no studies reporting on the effect of organic acids on external organs. Wang et al., (2010) reported that neither the gastrointestinal system nor the external organs were affected by the dietary inclusion of phenyllactic acid. The mode of action of feed additives on size of external organs is not well-understood and more research is needed to understand this effect.

4.4.4 Meat quality measurements

This study aimed to investigate whether the candidate zootechnical feed additives would alter meat quality traits. Meat pH is well known to have an effect on meat quality parameters such as flavour, tenderness, shelf-life and colour. A low pH has been associated with higher electrical conductivity and lightness during meat aging (Honiken, 1992; Peters, 1996). The group mean pH values in the current study ranged from 7.01 to 7.17 and there were no dietary differences ($P>0.05$) observed.

Organic acids exert antimicrobial effects on the feed before consumption and upon ingestion in the crop, gizzard and intestine (Cherrington et al., 1991), but do not seem to directly affect meat pH. Most of the biochemical processes, such as pH changes, should take place so that the animal muscle is converted into meat (Pearson, 1994). According to Jones and Grey, (1989), and Sams and Mills (1993), pH level in a live animal is approximately 7.4, while at the end of post-mortem ranges between 5.6 and 5.86.

Meat colour is a vital meat attribute that is normally measured in meat production chains so as to pinpoint quality deficiencies e.g., pale, soft and exudative meat. The objective is to meet the consumer demands with regards to product appearance (Resurreccion, 2003). The current study shows higher values for redness (Minolta a*) in meat from chickens fed antibiotic growth promoter (Table 4.4). These results are in contrast with the results found by Li et al., (2015) who found tylosin antibiotic growth promoter to have no effect on the redness of the meat. The lightness (Minolta L*) and the yellowness (Minolta b*) of meat was not influenced by diets. According to Sanudo (1992), meat quality is affected by the pH changes that take place during rigor mortis. Meat colour alterations, which mostly affect swine, such as PSE and DFD meat (dark, firm and dry), are not common in birds. In spite of that, changes in colour that are similar to PSE have been recorded in a number of studies (Northcutt, 1994). Interestingly, Allen et al. (1993) reported a positive correlation between meat colour and pH. Both lightness (L*) and yellowness (b*) showed negative correlation to pH, while redness had a positive correlation (Livingston and Brown, 1981).

The utility of probiotics in enhancing meat quality has been questionable due to several inconsistent results. Some studies indicated a positive effect of probiotic supplementation (Mahajan et al., 2000; Kim et al., 2005; Zhang et al 2005; Zhou et al., 2010), while others recorded no beneficial effects (Kim 2007; Kim & Yoon, 2008; Zhang et al., 2012). In the present study,

probiotics did not have much effect on the meat quality parameter; they only showed a negative effect on cooking loss and WHC. WHC and drip loss are some of the most important parameters of meat quality factors because some nutrients may be lost in the exudate, which may have a negative effect on the tenderness, flavour and juiciness of the meat (Chen et al., 2012). The other parameter to consider when looking at indicators of WHC is drip loss. The current study did not show any dietary effect on drip los,s but on WHC. The WHC result is in agreement with Kim et al., 2017, who observed an increase in the WHC of breast meat from chickens fed with a probiotic, although the detailed reasons for this are unknown. More studies are needed in order to understand the effect of probiotics on meat quality. Chelated mineral supplementation did not show any effects on meat quality. In agreement with our results, previous reports indicated that supplementing Mn result in no effect on the dressing percentage (Collins & Moran, 1999; Lü et al., 2007). In the present study the inclusion of Zn, Cu and Mn did not show significant ($P>0.05$) effect in all meat parameters beside for WHC and cooking loss.

4.5 Conclusions

The conclusion is that the inclusion of zootechnical feed additives does not affect most of the meat quality parameters. Most studies are focused on finding alternatives to AGPs in broiler diets, but the effects of those alternatives on meat quality are not known. More studies are needed to investigate the effects of these alternatives on meat quality

4.6 References

Allen, C.D., Fletcher, D.L., Northcutt, J.K. & Russell, S.M., 1998. The relationship of broiler breast color to meat quality and shelf-life. Poultry Sci. 77(2), 361-366.

Allen, C.D., Russell, S.M. & Fletcher. D.L., 1993. The relationship of broiler breast meat color and shelf-life and odor development. Poultry Sci. 76, 1042-1046.

Anadón, H., Denbow, D.M., Hohenboken, W.D., Emmerson, D.A., Graham, P.P. & Denbow, C.M., 2002. Biological, nutritional, and processing factors affecting breast meat quality of broilers. ProQuest Dissertations Publishing, Virginia Polytechnic Institute and State University.

Andersen, H.J., Oksbjerg, N., Therkildsen, M. & Young, J.F., 2005. Feeding and meat quality- a future approach. Meat Sci. 70, 543-554.

Award, W.A., Ghareeb, K., Abdel-Raheem, S., & Böhm, J. 2009. Effect of dietary inclusion of probiotic and synbiotic on growth performance, organ weights, and intestinal histomorphology of broiler chickens. Poultry Sci., 88 (1), 49–56.

Battula, V., Schilling, M.W., Vizzier-Thaxton, Y., Behrends, J.M., Williams, J.B. & Schmidt, T.B., 2008. The effects of low-atmosphere stunning and deboning time on broiler breast meat quality. Poultry Sci. 87 (6), 1202-1210.

Cai, L., Park, Y.S., Seong, S.I., Yoo, S.W. & Kim., 2015. Effects of rare earth elements-enrichments yeast on growth performance, nutrient digestibility, meat quality, relative organ weights, and extra microflora in broiler chickens., Livest. Sci. 172, 43-49.

Cavitt, L.C., Meullenet, J.F., Gandhapuneni, R.K., Youm, G.W. & Owens, C.M., 2005. Rigor development and meat quality of large and small broilers and the use of Allo-Kramer shear, needle puncture, and razor blade shear to measure texture. Poultry Sci. 84 (1), 113-118.

Chen, H., Dong, X., Yao, Z., Xu, B., Zhen, S., Li, C. & Li, X., 2012. Effects of pre-chilling parameters on water-holding capacity of chilled pork and optimization of pre-chilling parameters using response surface methodology. J. Anim. Sci. 90, 2836-2841.

Cherrington, C.A., Chopra, I. & Hinton, M., 1991. Acid treatment of feed for the control of Salmonella infections in poultry. Veterinary Annual. 31, 90-95.

Collins, N.E. & Moran Jr, E.T., 1999. Influence of supplemental manganese and zinc on live performance and carcass quality of broilers. J Appl Poultry Res. 8 (2), 222-227.

Deatherage, F.E., 1963. The effect of water and inorganic salts on tenderness. In Proceedings meat tenderness symposium (pp. 45-68).

Delezie, E., Swennen, Q., Buyse, J. & Decuypere, E., 2007. The effect of feed withdrawal and crating density in transit on metabolism and meat quality of broilers at slaughter weight. Poultry Sci. 86, 1414-1423.

Emmans, G.C., 1981. A model of the growth and feed intake of ad libitum fed animals, particularly poultry. In: Computers in animal production. Eds: Hillyer, G.M., Whittemore, C.T. & Gunn, R.G., Animal Production, Occasional publication 5, London, pp.103-110.

Fletcher, D.L., 2002. Poultry meat quality. World Poultry Sci. J. 58, 131-146.

Grau, R. & Hamm, R., 1957. Biochemistry of meat hydration. Adv. Food Res. 10, 356-359.

Heckert, R.A., Estevez, I., Russek-Cohen, E. & Pettit-Riley, R., 2002. Effects of density and perch availability on the immune status of broilers. Poultry Sci. 81, 451-457.

Honikel, K-O., 1992. Qualitätsprodukte erfordern geeignete Messmethoden. In: Qualitätssicherung im Fleischbereich. Kulmbacher Reihe, 11, 1-18.

Jones, J.M. & Grey, T.C., 1989. Influence of processing on product quality and yield. In: Processing of poultry. Ed.: Mead, G.C., Elsevier Applied Science, New York, pp. 127-130.

Karacay, N., Ocak, N., Sarica, M. & Erener, G., 2008. Effect of carbohydrate supplementation provided through drinking water during feed withdrawal on meat and liver colours in broilers. J. Sci. Food Agric. 88, 479-484.

Kim, K.S., Lee, J.H., Shin, M.S., Cho, M.S., Kim, Y.P., Cho, S.K. & Kang, Y.J., 2005. Effect of dietary probiotics supplementation contained with astaxanthin produced by Phaffia rhodozyma on the productivity and meat quality of ducks. Kor. J. Poult. Sci. 32, 73-80.

Kim, Y.J. & Yoon, Y.B., 2008. Effect of the feeding probiotics, illite, activated carbon, and hardwood vinegar on the meat quality and shelf-life in chicken thigh. Kor. J. Food Sci. Anim. Resour. 28, 480-485.

Kim, Y.J., 2007. Effect of dietary supplementation with probiotics, illite, active carbon and hardwood vinegar on the performance and carcass characteristics of broiler. Kor. J. Poult. Sci. 34, 165-172.

Kim, Y.J., Park, W.Y. & Choi, I.H., 2010. Effects of dietary α-tocopherol, selenium, and their different combinations on growth performance and meat quality of broiler chickens. Poultry Sci. 89 (3), 603-608.

Li, H.L., Zhao, P.Y., Lei, Y., Hossain, M.M., Kim, I.H., 2015. Phytoncide, phytogenic feed additive as an alternative to conventional antibiotics, improved growth performance and decreased excreta gas emission without adverse effect on meat quality in broiler chickens Livestock Sci. 181, 1-6

Livingston, D.J. & Brown, W.D., 1981. The chemistry of myoglobin and its reactions. Food Technol. 35, 244-252.

Lu, L., Luo, X.G., Ji, C., Liu, B. & Yu, S.X., 2007. Effect of manganese supplementation and source on carcass traits, meat quality, and lipid oxidation in broilers 1. J Anim Sci. 85(3), 812-822.

Lyon, B.G., Smith, D.P., Lyon, C.E. & Savage, E.M., 2004. Effects of diet and feed withdrawal on the sensory descriptive and instrumental profiles of broiler breast fillets. Poultry Sci. 83, 275-281.

Mahajan, P., Sahoo, J. & Panda, P.C,. 2000. Effect of probiotic (Lacto-Sacc) feeding, packaging methods and season on the microbial and organoleptic qualities of chicken meat balls during refrigerated storage. J. Food Sci. Technol. 37, 67-71.

Moreira, J., Mendes, A.A., Garcia, E.A., Garcia, R.G., & Almeida, I.C.L. 2001. Efeito do uso do probiótico sobre o desempenho e rendimento de carcaça em frangos de corte. In: XXXVIII Reunião Anual da SBZ, Piracicaba. Anais, Piracicaba, pp. 852-854.

Northcutt, J.K., Buhr, R.J., Berrang, M.E. & Fletcher, D.L., 2003. Effects of replacement finisher feed and length of feed withdrawal in broiler carcass yield and bacteria recovery. Poultry Sci. 82, 1820-1824.

Offer, G., & Knight, P. (1988b). The structural basis of water-holding capacity in meat. Part 2: drip losses. In R. Lawrie (Ed.). Developments in meat science (Vol. 4, pp. 173–243). London: Elsevier Science Publications.

Paryad, A. & Mahmoudi, M., 2008, Effect of different levels of supplemental yeast (Saccharomy cescerevisiae) onperformance, blood constituents and carcass characteristics of broiler chicks. Afr. J. Agric. Res. 3, 835-842.

Pearson, A.M., 1994. La fucion muscular y los cambios post mortem. In: Ciencia de la carne y de los productos carnicos. 2nd edn. Eds: Price, J.R., & Scheweigert, B.S., Zaragoza, Acribia, p. 273.

Pelicano, E.R.L., De Souza, P.A., De Souza, H.B.A., Oba, A., Norkus, E.A., Kodawara, L.M. & De Lima, T.M.A. 2003. Effect of different probiotics on broiler carcass and meat quality. Revista Brasileira de Ciência Avícola, 5(3), 207-214.

Peters, H. 1996. Qualitätsbewertung von Fleisch und Fleischerzeugnissen. In: Fleischtechnologie. Ed. Sielaff H., Behr's Verlag, Hamburg, Germany, pp. 559-606.

Resurreccion, A.V.A., 2003. Sensory aspects of consumer choices for meat and meat products. Meat Sci. 66, 11-20.

Sams, A.R. & Mills, K.A. 1993. The effect of feed withdrawal duration on the responsiveness of broiler pectoralis to rigor mortis acceleration. Poultry Sci. 72, 1789-1796.

Sañudo, C., Santolaria, P., Sierra, I., Alcalde, M.J. & Touraille, C., 1992. Sensory meat characteristics from light lamb carcasses. In: Internacional Congress of Meat Science and Technology, Clermont-Ferrant, France. Proceedings... Ferrant: Clermont (pp. 277-280).

Savell, J.W., Cross, H.R., Francis, J.J., Wise, J.W., Hale, D.S., Wilkes, D.L. and Smith, G.C., 1989. National consumer retail beef study: Interaction of trim level, price and grade on consumer acceptance of beef steaks and roasts. J Food Quality 12 (4), 251-274.

Skinner, J.T., Izat A.L. & Waldroup, P.W., 1991. Research note, Fumaric acids enhances performance of broiler chickens. Poultry Sci. 70(6), 1444-1447.

Solomon, M.B., Van Laack, R.L.J.M. & Eastridge, J.S. 1998. Biophysical basis of pale, soft exudative (PSE) pork and poultry muscle: A review. J Muscle Foods 9, 1–11.

Sosnicki, A.A, & Wilson, B.W., 1991. Pathology of turkey skeletal muscle, Implications for the poultry industry. Food Structure. 10, 317-326.

Van Laack, R.L.J.M., Liu, C.H., Smith, M.O. and Loveday, H.D., 2000. Characteristics of pale, soft, exudative broiler breast meat. Poultry Sci. 79(7), 1057-1061.

Varel, V.H., 2002. Livestock manure odor abatement with plant-derived oils and nitrogen conservation with urease inhibitors: A review 1. J. Animal Sci. 80 (E-Suppl_2), E1-E7.

Vargas, J.G., Toledo, R.S., Albino, L.F.T, Rostango, H.S., Oliveira, J.E. & Carvalho, D.C.O., 2002. Características de carcaça de frango de corte, submetidos a rações contendo probióticos, prebióticos e antibióticos. In: XXXIX Reunião Anual da SBZ, Recife. Anais, Recife, CD ROM.

Visek, W.J., 1978. The mode of growth promotion by antibiotics. J. Anim. Sci. 46, 1447-1469.

Zhang, A.W., Lee, B.D., Lee, S.K., Lee, K.W., An, G.H., Song, K.B. & Lee, C.H., 2005. Effects of yeast (Saccharomyces cerevisiae) cell components on growth performance, meat quality, and ileal mucosa development of broiler chicks. Poultry Sci. 84, 101-1021.

Zhang, Z.F., Cho, J.H. & Kim, I.H., 2013, Effects of Bacillus subtilis UBT-MO2 on growth performance, relative immune organ weight, gas concentration in excreta, and intestinal microbial shedding in broiler chickens. Livest. Sci. 155, 343-347.

Zhang, Z.F., Zhou, T.X., Ao, X. & Kim, I.H., 2012. Effects of β-glucan and Bacillus subtilis on growth performance, blood profiles, relative organ weight and meat quality in broilers fed maizesoybean meal based diets. Livest. Sci. 150, 419-424.

Zhou, X., Wang, Y., Gu, Q. & Li, W., 2010. Effect of dietary probiotic, Bacillus coagulans, on growth performance, chemical composition, and meat quality of Guangxi Yellow chicken. Poultry Sci. 89, 588-593.