

**EFFECTS OF ESSENTIAL OIL, PROBIOTIC, PALM KERNEL FATTY
ACID DISTILLATE, OPTIGUT, OR SUNFLOWER WHOLE SEEDS AS
ALTERNATIVES TO ANTIBIOTIC GROWTH PROMOTERS ON
BROILER PERFORMANCE**

by

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PREFACE AND DECLARATION

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DEDICATION

I dedicate this dissertation to Mlungisi Tshonaphi (My brother).

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LIST OF ABBREVIATION

ADG	-Average daily gain
BA	-Butyric acid
BW	-Body weight
<i>C. perfringens</i>	- <i>Clostridium perfringens</i>
EO	-Essential oils
FCR	-Feed conversion ratio
FI	-Feed intake
FOS	-Fructo-oligosaccharide
GIT	-Gastro-intestinal tract
MCFA	-Medium chain fatty acids
MOS	-Mannan-oligosaccharide
NE	-Necrotic enteritis
PKFAD	-Palm kernel fatty acid distillate
SBP	-Sugar beet pulp

ABSTRACT

The increasing consumer and legislation pressure to phase out the antibiotic growth promoters (AGPs) in the broiler industry has prompted researchers to find suitable alternatives to AGPs that will improve broiler performance at levels comparable to AGPs. There were three trials that were conducted in the present study. The aim of the first trial was to evaluate the effects of supplementing broiler diets with Oligo essential (essential oil) or AGPs on growth performance and caecal *Clostridium perfringens* (*C. perfringens*) counts of broilers from 0 to 33 days of age, reared in a commercial farm. A total of ten broiler houses were used for the trial. Five houses were designated for broilers receiving feed supplemented with AGPs and other five houses for broilers receiving feed supplemented with Oligo Essential. The houses were used as experimental units. The control houses (1, 2, 3, 4, and 5) were paired with trial houses (7, 8, 9, 10, and 11). The chicks placed in paired houses had the same parent flock age, were placed on the same day and also slaughtered at the same age. A total of 300 000 day old broiler chicks (Cobb 500) of mixed sex were used in the trial and the stocking density per house was 22.10 birds/m². In the period of 0 to 33 days of age, it was observed that the broilers that were fed diets supplemented with Oligo Essential had a significantly poorer feed conversion ratio (FCR) and higher feed intake (FI) when compared to broilers that were fed diets containing AGPs. However, no effect of dietary treatment was seen on the body weight (BW). The caecal *C. perfringens* counts at 9 and 30 days of age were unaffected by dietary treatment. In conclusion, supplementing broiler diets with Oligo Essential had negative effects on broiler performance in the present study.

In the second trial, the objective was to determine the effects of *Lactobacillus* based probiotic or AGPs on broiler performance in a commercial farm. A total of six broiler houses were used for the trial. Three houses were designated for broilers receiving feed supplemented with AGPs and other three houses for broilers receiving feed without AGPs, but all the day-old chicks were sprayed with *Lactobacillus* based probiotic at the hatchery. The dosing volume was 10ml per 100 chicks. The houses were used as experimental units. The control houses (1, 2, and 3) were paired with trial houses (4, 5, and 6). The chicks placed in paired houses had the same parent flock age, were placed on the same day and also slaughtered at the same age. A total of 180 000 day old broiler chicks (Cobb 500) of mixed sex were used in the trial and the stocking density per house was 20.80 birds/m². It was noted that there was no significant difference in BW, FCR, FI and mortality between the treatments at 33 days of age. Therefore, it was concluded that the *Lactobacillus* based probiotic demonstrated feasibility of being a substitute for AGPs as the broiler performance was comparable to broilers that received diets supplemented with AGPs.

In the third trial, the objective was to investigate the effects of Optigut, palm kernel fatty acid distillate and sunflower whole seeds on broiler performance, organs weights, intestinal length, digesta pH and caecal microbial profile. A total of 3360 Cobb 500 day old broiler chicks were randomly distributed into 48 pens. There were six dietary treatments for the trial: (i) Negative control with no AGPs; (ii) Negative control supplemented with AGPs; (iii) Negative control supplemented with Palm kernel fatty acid distillate at 2.5%; (iv) Negative control supplemented with Optigut at 0.4% in the starter, 0.2% in the grower and 0.1% in the finisher; (v) Negative control supplemented with Sunflower whole seeds at 4.0%; (vi) Negative control supplemented with Palm kernel fatty acid distillate (2.5%) and Sunflower whole seeds (4.0%).

It was observed that there was no significant treatment effect on broiler performance parameters, organs weights, intestinal length, digesta pH and caecal microbial profile at 35 days of age. The results of the study suggest that the trial was conducted in a hygienic environment, therefore, it was recommended to conduct challenge studies to further investigate the effects of these alternatives to AGPs.

CHAPTER 1

GENERAL INTRODUCTION

Background

Antibiotics have played a vital role in poultry production as growth and health promoters in the last few decades. Antibiotics are used by the poultry industry for three purposes: (i) to treat sick animals; (ii) to prevent infections; (iii) and to improve feed conversion and growth rate. The use of antibiotics for sub-therapeutic purposes to improve broiler efficiency is anticipated to decrease in the future due to public concerns of the potential transfer of antibiotic resistance from chicken meat products to humans (Kelly *et al.*, 2004). Based on this concern, the European Union Commission banned the use of antibiotic growth promoters (AGPs) in 2006 (Castanon, 2007). The regulation that was enforced to ban the use of AGPs in European countries has forced the countries that are interested in exporting chicken products to European countries to stop using AGPs (Aristimunha *et al.*, 2016). It is very clear that the use of AGPs may be discontinued worldwide due to consumer pressure (Dibner and Richards, 2005). Hence, poultry producers will be forced to phase out AGPs.

Phasing out AGPs has brought problems such as increased FCR (Engster *et al.*, 2002) and a rise in intestinal health problems often referred to as dysbacteriosis (Teirlynck *et al.*, 2011), which is characterised by reduced growth rate, diarrhoea with undigested feed particles and also poor flock uniformity (Teirlynck *et al.*, 2011). In addition, inconsistent literature research regarding the use of alternatives to AGPs, high costs of AGPs replacements and production loss are major challenges that poultry producers are facing.

In 2014 the World Health Organisation stated that use of antibiotics for sub-therapeutic purposes was a public health issue and a global coordinated action plan is required to decrease the usage of AGPs. An integrated approach that incorporate feed, farm and health management strategies would be essential to phase out AGPs. Management changes such as strict biosecurity measures, decreasing stoking density, increasing use of vaccines and also use alternatives to AGPs might play a crucial role to improve broiler performance at levels comparable to AGPs.

The aim of this study was to investigate alternatives to AGPs that will improve performance of broiler chickens at levels comparable to AGPs and also prevent colonisation by pathogens such as *Clostridium perfringens*.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Consumer pressure and legislation is forcing poultry producers to grow broiler chickens without AGPs. This calls for the need to find suitable alternatives to AGPs that will improve performance of broiler chickens at levels comparable to AGPs. A wealth of work has been conducted worldwide to find suitable replacements for AGPs. The effective use of alternatives to AGPs to improve broiler performance depends on the degree of understanding their mode of action. The AGPs and their alternatives may have different modes of action. The AGPs reduce the total microbial load in the GIT and that results in more nutrients utilised by the host to enhance the growth rate and feed conversion ratio (Miles *et al.*, 2006). In contrast, alternatives to AGPs modify the microbial profile in the GIT by inhibiting the growth of pathogenic bacteria while promoting the growth of beneficial bacteria. It is essential that the alternatives to AGPs must be used in a way that compliments their mode of action in order to improve the growth rate and feed conversion ratio.

This review will discuss the description and proposed mode of action of the following seven kinds of alternatives to AGPs. Namely; Probiotics, prebiotics, synbiotic, butyric acid, essential oil, high dietary fibre and medium chain fatty acids. In addition, the effects of these AGPs replacements on broiler performance will also be covered in this study.

2.2 Antibiotic Growth Promoters

Interestingly, AGPs have been used for many decades in broiler production but the mechanism by which they enhance the performance of broilers is not exactly known (Aristimunha *et al.*, 2016). It has been reported that AGPs do not have growth promoting effects in germ-free chickens (Feighner and Dashkevicz, 1987). A wealth of research has been conducted in order to determine the mechanism of AGPs and researchers concur on the following proposed mode of action of AGPs: (i) reduce microbial load in the gastro-intestinal tract; (ii) reduce use of nutrients by micro-organisms since competing micro-organisms are reduced; (iii) improve absorption of nutrients due to thinner small intestinal epithelium; (iv) decrease pathogenic micro-organisms that cause subclinical infections (Feighner and Dashkevicz, 1987; Knarreborg *et al.*, 2004).

2.3 Probiotics

Probiotics are regarded as one of the suitable replacements to antibiotics as they have a potential to reduce the pathogenic bacteria in broilers and subsequent contamination of chicken meat. Probiotics are defined as live microbial feed additives, which positively influence the intestinal microbiota of the host (Yun *et al.*, 2017). *Lactobacillus*, *Enterococci*, *Bacillus*, *Streptococcus*, *Bifidobacterium*, *Lactococcus*, and *Saccharomyces cerevisiae* are the microbial species that have been used as probiotics in poultry feed (Salim *et al.*, 2013). However, the use of these microbial species must be able to survive environmental conditions during storage and processing of feed (Lan *et al.*, 2003).

The proposed mechanism of action of probiotics in poultry include: competitive exclusion of pathogenic micro-organisms in order to maintain a beneficial microbial population; suppressing

the growth of *C. perfringens* to control necrotic enteritis; enhancement of the bird's natural immune response against pathogenic bacteria through stimulating the immune system that is linked with the gut; increasing epithelial integrity which results in better utilisation of nutrient; production of metabolites that have antimicrobial activity; compete with pathogenic micro-organisms for nutrient utilisation; (Rolfe, 2000; La Ragione *et al.*, 2004; Awad *et al.*, 2009; Tactacan *et al.*, 2013; Schneitz *et al.*, 2016). Furthermore, probiotics have pH reducing properties (Ghasemi *et al.*, 2016).

Mountzouris *et al.* (2010) conducted a study to investigate the effects of different inclusion levels of a 5-bacterial species probiotic product (*Lactobacillus reuteri*, *Enterococcus faecium*, *Bifidobacterium animalis*, *Pediococcus acidilactici* and *Lactobacillus salivarius*) in broiler diets on growth performance. The broilers were fed one of five dietary treatments (negative control with no additive, negative control supplemented with 10^8 CFU/kg probiotic, negative control supplemented with 10^9 CFU/kg probiotic, negative control supplemented with 10^9 CFU/kg probiotic and positive control with avilamycin at 2.5 mg/kg as AGPs). Broilers on the lowest dose of probiotic were shown to have a significantly higher BW compared to other treatment groups at 42 days of age. The AGPs group had a significantly higher BW than a negative control, intermediate and highest dose of probiotics. It was also noted that the lowest dose of probiotic and AGPs groups had the same FCR and were significantly better compared to other treatments. FI was, however, not affected by dietary treatments.

Santoso *et al.* (1995) determined the effects of *Bacillus subtilis* (*B. subtilis*) on BW and FCR. Broilers were fed a diet containing 10 or 20 g/kg of *B. subtilis* and their performance was compared to broilers that were fed a negative control diet. BW was not affected by the treatment groups, however, the addition of *B. subtilis* resulted in a significant improvement in FCR when compared to the negative control group.

Tactacan *et al.* (2013) conducted an NE challenged study to determine the adequate inclusion level of *B. subtilis* (1×10^5 or 1×10^6 CFU/g) that could reduce the negative effects of NE on broiler performance. There were five treatment groups: (i) negative control + infected; (ii) negative control + non-infected; (iii) AGPs + infected; (iv) 1×10^5 CFU/g *B. subtilis* + infected and (v) 1×10^6 CFU/g *B. subtilis* + infected. The negative control group that was not infected was shown to have a better performance in terms of growth rate and FCR than the other four groups that were infected. There was no significant difference on ADG between broilers fed diets containing a high dose of *B. subtilis* (1×10^6 CFU/g) and those fed diets containing AGPs. However, broilers on high dose of *B. subtilis* showed a significant improvement in FCR compared to broilers that were fed a diet containing AGPs.

Olnood *et al.* (2015b) determined the effectiveness of different methods for administering a probiotic (*Lactobacillus johnsonii*) to broilers on growth performance. There were seven treatments for the trial (negative control diet with no additive, negative control diet supplemented with $>10^6$ CFU/g *Lactobacillus johnsonii*, drinking water was supplemented with $>10^6$ CFU/ml *Lactobacillus johnsonii*, $>10^8$ CFU/g *Lactobacillus johnsonii* sprayed on litter, $>10^6$ CFU/g

Lactobacillus johnsonii gavage orally and positive control diet with zinc bacitracin at 50mg/kg). The results exhibited that the different methods of administering *Lactobacillus johnsonii* had no significant effect on BW, FCR and FI at 35 days of age.

Ghasemi and Taherpour (2013) observed that BW and FCR of broilers were positively affected when a probiotic (*Enterococci*) was added in broiler diets. A significant improvement in BW as well as FCR was seen in broilers that were fed diets containing a probiotic compared to those that were fed a negative control diet.

There is inconsistency with regards to the effect of probiotics on broiler performance. The differences in performance could be ascribed to factors such as probiotic type (e.g., *Lactobacillus*, *Enterococci*, *Bacillus*, *Streptococcus* or *Bifidobacterium*) and dosage (e.g., lowest or highest), rearing conditions (e.g., hot or cold areas), health status of the birds, breed (e.g., Cobb or Ross), sex (e.g., males, females or mixed sex), composition of the diets (e.g., different inclusion levels of enzymes or no enzymes added) and management practices (e.g., temperature, humidity or stocking density) (Houshmand *et al.*, 2011; Nunes *et al.*, 2012).

2.4 Prebiotics

Prebiotics are non-digestible feed ingredients that improve the microbial balance of the host through its selective effects on the intestinal microbiota (Al-Owaimer *et al.*, 2014). Oligosaccharides are the main components of prebiotics and they can be based on any of the different hexose monosaccharides such as mannose, galactose, glucose and fructose (Durst, 1996).

The mode of action of prebiotics include selective stimulation of the growth of beneficial bacteria such as *Bifidobacteria* and *Lactobacilli* in the gut, hence, improving the intestinal integrity (Gibson and Roberfroid, 1995). Prebiotics also suppresses the growth of pathogens by providing them a competitive binding site (Kim *et al.*, 2011). It is vitally important to note that prebiotics only stimulate the growth of beneficial bacteria that are present in the gut of the host (Al-Baadani *et al.*, 2016). This implies that the effectiveness of prebiotics is dependent on the population of beneficial micro-organisms in the GIT.

Kim *et al.* (2011) determined the effects of adding mannan-oligosaccharide (MOS) and fructo-oligosaccharide (FOS) in broiler diets on broiler performance parameters and intestinal populations of *C. perfringens*, *E.coli* and *Lactobacilli*. The broilers were fed one of six feed treatments (negative control, AGPs, FOS (0.25%), FOS (0.5%), MOS (0.025) and MOS (0.05%). Broilers on AGPs, FOS (0.25%) or MOS (0.05%) showed significantly higher BW in comparison to other treatment groups at 28 days of age. The FCR, FI and mortality were however not affected by dietary treatment. In addition, broilers on AGPs, FOS (0.25%) or MOS (0.05%) showed a significant decrease in *C. perfringens* and *E.coli* populations than the other treatment groups. It was also seen that the *Lactobacilli* population was significantly higher in broilers that were fed diets containing FOS (0.25), MOS (0.025) or MOS (0.05) compared to other treatment groups. This shows that a reduction in harmful microbial population is an indicative of a healthy gut which would subsequently improve nutrient utilization and absorption and would thereby improve the growth rate of broilers.

Wang *et al.* (2016) determined the effects of prebiotics, probiotics and the combination of prebiotics and probiotics on performance parameters and resident *Lactobacillus* of male broilers. The broilers were fed one of the following five dietary treatments: (i) negative control with no additives; (ii) negative control supplemented with a prebiotic consisting a MOS and β -glucans at 170 and 250 g/ton, respectively; (iii) negative control supplemented with a probiotic containing *B. subtilis* strain at 300 000 CFU/g; (iv) negative control supplemented with the combination of the above mentioned prebiotic and probiotic products and (v) positive control containing AGPs (bacitracin, nicarbazin and narasin at 50, 54 and 54 g/ton). It was reported that the addition of prebiotics (MOS and β -glucans) in broiler diets resulted in a significantly poorer FCR and lower BW in comparison to broilers that were fed diets containing AGPs at 42 days of age. It was also seen that the inclusion of prebiotics significantly increased the *Lactobacilli* population in the ileal of broilers.

Yang *et al.* (2008) conducted a study to investigate the effects of supplementing MOS in broiler diets on growth performance of broilers. The broilers were fed one of four dietary treatments (negative control with no additive, positive control with zinc bacitracin as AGPs, negative control supplemented with 1g/kg of MOS and negative control supplement with 2g/kg of MOS). The results showed that there was no significant treatment effect on BW, FCE, FI or mortality at 35 days of age.

There is still a large variation in terms of the effectiveness of prebiotics compared to antibiotics when it comes to improving the broiler performance (Ajuwon *et al.*, 2016). The inconsistent effect

of prebiotics on broiler performance might be attributable to different inclusion levels and sources of the products added in broiler diets (Fowler *et al.*, 2015).

2.5 Synbiotic

Synbiotic is a combination of prebiotic and probiotic (Patterson and Burkholder, 2003) which might have synergetic effects on the gut of chickens (Ghasemi *et al.*, 2016). Synbiotic has been reported to have positive effects on the host by improving the survival and persistence of live microbial dietary supplements in the gastro-intestinal track of chickens (Awad *et al.*, 2009). Synbiotic changes the intestinal microbiota by stimulating the growth of beneficial bacteria and simultaneously reducing colonisation by pathogens (Patterson and Burkholder, 2003).

Ghasemi and Taherpour (2013) evaluated the effects of synbiotic on BW, FCR and FI of broiler chickens. Broilers were fed one of four dietary treatments (control, probiotic (*Enterococcus*, prebiotic (fructo-oligosaccharides) and synbiotic (mixture of *Enterococcus* and fructo-oligosaccharides). Broilers that were fed diets containing synbiotic showed significantly higher BW and better FCR compared to other treatment groups at 42 days age. It was also shown that the FI was not influenced by dietary treatments. It was recommended that synbiotic could be a suitable replacement for AGPs in broiler production.

Dizaji *et al.* (2012) also observed that the addition of synbiotic in broiler diets resulted in a significant improvement in FCR and BW at 42 days of age compared to the control, prebiotic and probiotic groups when broilers were fed one of five dietary treatments ((i) control diet, (ii) control

diet supplemented with prebiotic (MOS) at 1 kg /ton, (iii) control diet supplemented with probiotic (Protexin) at 150 g/ton, (iv) control diet supplemented with synbiotic (Amax4x) at 1 kg/ton and (v) control diet supplemented with acidifier (Globacid) at 2 litre/ton. According to Al-Baadani *et al.* (2016) the improved broiler performance with the addition of synbiotic could be ascribed to increased intestinal villi height and balanced microbiota in the GIT that enhance absorption of nutrients.

2.6 Butyric Acid

Butyric acid (BA) is a short chain fatty acid that is considered as potential replacement for AGPs (Leeson *et al.*, 2005). It has been reported to have antimicrobial properties (van Immerseel *et al.*, 2005; Fernandez–Rubio *et al.*, 2009). However, the antimicrobial activity of BA is pH dependent, as BA has pH reducing properties in different parts of the GIT of broilers (Panda *et al.*, 2009). The reduced pH will create favourable conditions in the GIT for the beneficial micro-organisms to grow while simultaneously hindering the growth of pathogenic micro-organisms which are acid-intolerant (Adil *et al.*, 2010). The reduction of intestinal pathogens result in decreased competition between the pathogens and host for nutrients and also decrease the growth depressing toxins produced by pathogens (Adil *et al.*, 2011). This means more nutrients will be available to the host for absorption. It has also been reported that BA plays a very important role in the development of epithelial cells in the intestines (Dalmasso *et al.*, 2008; Guilloteau *et al.*, 2010), which improves the utilisation of nutrients by the host. In addition, BA has a positive effect on intestinal histomorphology, as it has been reported to significantly increase the villus height in the small intestines (Panda *et al.*, 2009; Adil *et al.*, 2010). An increase in villus height increases the surface area for nutrient absorption, thus, decreasing the amount of substrate that will be fermented by micro-

organisms in the hindgut (Qaisrani *et al.*, 2015). This means that there will be less nutrients available to be utilised by pathogenic bacteria.

Researchers have reported inconsistent results regarding the effect of BA on broiler performance parameters (Table 2.1). Leeson *et al.* (2005) observed that the broiler performance was not affected by the addition of BA into broiler diets. Broilers were fed one of four feed treatments (negative control, virginiamycin, 0.2 % BA and 0.4% BA). No significant treatment effect was seen on BW, FCR, FI and mortality. The lack of response with the addition of either virginiamycin or BA might be due to broilers being healthy and there were no environmental challenges.

Kaczmarek *et al.* (2016) observed a linear effect of BA addition on FCR when broilers were fed one of four dietary treatments (control, 0.2, 0.3, and 0.4 g/kg BA). Birds on highest dose of BA were shown to have better FCR than the other treatment groups. The BW and FI were however not influenced by the feed treatments. In the same experiment, broilers that were fed diets containing BA were shown to have higher villus height compared to the control. The improved FCR of broilers on BA could be ascribed to improved digestion of nutrients (Qaisrani *et al.*, 2015) and increased intestinal absorptive area, consequently facilitating nutrient absorption. In addition, the positive effects of BA supplementation might also be attributed to their antimicrobial properties.

Table 2. 1 Effect of butyric acid and antibiotic growth promoter on body weight (BW), feed conversion ratio (FCR), body weight (BW) and feed intake (FI) in broilers

Treatment	Age (d)	BW (g)	FCR	FI (g/d)	Reference
Control		2546	1.83	4659	
Virginiamycin		2515	1.81	4551	
0.2% butyric acid	0-42	2605	1.80	4690	Leeson <i>et al.</i> , 2005
0.4% butyric acid		2554	1.80	4588	
Control		2616	1.66	4336	
0.2 g/kg butyric acid	0-42	2696	1.64*	4409	Kaczmarek <i>et al.</i> , 2016
0.3 g/kg butyric acid		2741	1.59*	4348	
0.4 g/kg butyric acid		2699	1.58*	4207	

* Means were significantly different compared to the control (P<0.05).

2.7 Essential oils

Essential oils (EO) are natural plant based products which have both antibacterial (Thanissery *et al.*, 2014) and antioxidant activities (Hoffman-Pennesi and Wu, 2010). Phenolics and alkaloids are active components of plant EO (Thanissery *et al.*, 2014). Phenolics are hydrophobic which allows them to adhere to the lipid bilayer of the cytoplasmic membrane to cause ion leakage and make micro-organisms less virulent (Frankic *et al.*, 2009). It has been reported that EO have inhibitory effects against gram-negative bacteria such as *Salmonella* (Machado Junior *et al.*, 2014) as well as gram-positive bacteria such as *C. perfringens* (Mitsch *et al.*, 2004). Including EO in broiler diets might promote a healthy environment in the GIT by inhibiting the growth of pathogenic bacteria, hence, the host will be less exposed to toxins of bacterial origin (Frankic *et al.*, 2009). The composition of EO is very variable due to the following factors: (i) biological factors such as plant species and growing region (Karamian *et al.*, 2015); (ii) manufacturing process - EO can be produced by solvent extraction (Mwaniki *et al.*, 2015) or steam distillation (Sousa *et al.*, 2002); (iii) storage conditions such as temperature and time (Pratiwi *et al.*, 2016).

Ertas *et al.* (2005) determined the effects of supplementing EO (oregano, clove and anise) on broiler performance and the birds were fed one of five dietary treatments (negative control with no additives, negative control supplemented with EO at 100 ppm, negative control supplemented with EO at 200ppm, negative control supplemented with EO at 400 ppm or positive control with avilamycin (0.1%). It was shown that the addition of EO at 200 ppm significantly improved the ADG and FCR compared to other treatments at 35 days of age. It was noted that the ADG of broilers that were fed positive control diets was significantly better than those fed negative control, 100 ppm EO and 400 ppm EO. It also was seen that there was no significant difference in FCR between the 100 ppm EO group and positive control group and these two groups had a significantly better FCR compared to the negative control and 400 ppm EO groups.

Amerah *et al.* (2012) conducted a *Salmonella* challenge study to determine the effects of EO on performance and *Salmonella* Heidelberg proliferation on broilers. There were 5 treatments for the trial (negative control diet with no additives, negative control diet and birds were challenged with *Salmonella* Heidelberg (5 x 10⁵, negative control diet supplemented with EO (cinnamaldehyde and thymol) at 100 g/ton and birds were challenged with *Salmonella* Heidelberg (5 x 10⁵ CFU/ml), negative control diet supplemented with xylanase (2 000 U/Kg) and birds were challenged with *Salmonella* Heidelberg (5 x 10⁵ CFU/ml and negative control diet supplemented with EO (100 g/ton) and xylanase (2 000 U/Kg) and birds were challenged with *Salmonella* Heidelberg (5 x 10⁵ CFU/ml). The addition of EO in broiler diets was shown to significantly improve the FCR and BW of broilers that were challenged with *Salmonella* Heidelberg compared to the control and challenged control groups at 42 days of age. It was also seen that the EO group significantly

reduced the *Salmonella* Heidelberg positive caecal samples compared to the challenged control group. It was concluded that supplementing broiler diets with EO could be used improve broiler performance and also control *Salmonella* levels in broiler production.

Khattak *et al.* (2014) found that feeding broilers a blend of EO (basil, caraway, laurel, lemon, oregano, saga, tea and thyme) had a beneficial effect on broiler performance. Broilers were fed one of the six dietary treatments (negative control, 100, 200, 300, 400 or 500g/t of EO). The results showed that all broilers that were fed diets containing EO regardless of inclusion level had a significantly higher BW and better FCR than a control group at 42 days of age. The FI was not significantly influenced by dietary treatment. It was also noted that the BW gain or FCR of broilers was not dependent on dose of EO, as there were no differences in performance parameters between the lowest and highest dose of EO. It was recommended that the inclusion of 100g/t of EO would be sufficient to improve the performance of broilers.

2.8 High dietary fibre in broiler diets

The inclusion of raw materials that are rich in fibre in broiler diets has been regarded as an alternative nutritional strategy to replace AGPs (Gonzalez-Alvarado *et al.*, 2007). Traditionally, the use of fibre sources in broiler diets is believed to have negative effects on broiler performance as fibre has been regarded as an anti-nutritional factor that suppress the FI and depress growth rate of broiler chickens (Mateos *et al.*, 2012).

Fibre is classified into two categories which are soluble and insoluble (Sarikhani *et al.*, 2010). Insoluble fibre encourages gizzard development and functionality (Jimenez-Moreno *et al.*, 2010) and also reduces the pH in the gizzard (Jimenez-Moreno *et al.*, 2009a). Furthermore, improved gizzard activity stimulates hydrochloric acid (HCl) production and favours gastroduodenal refluxes which results in an improvement in nutrient digestibility (Mateos *et al.*, 2012). This results in acidification of the GIT which would create unfavourable conditions for pathogens.

Soluble fibre has a higher water holding and swelling capacity that results in the accumulation of digesta in the gizzard (Gonzalez-Alvarado *et al.*, 2008). In addition, soluble fibre increases digesta viscosity in the GIT and subsequently reduces digestion and absorption of nutrients (Smits *et al.*, 1997). The attributes of soluble fibre makes it less effective in developing and improving the functionality of the gizzard.

Research has shown that the use of moderate amount of fibre sources in broiler diets had positive effect on broiler performance (Table 2.2). Gonzalez-Alvarado *et al.* (2007) reported that the addition of 3% of either oat or soy hulls in broiler diets resulted in a significant higher growth rate and better FCR compared to the broilers that were fed a control diet, whilst FI was not affected by dietary treatment. It was also noted that incorporating oat or soy hulls in broiler diets showed significant reduced pH of the gizzard digesta compared to the control. This suggests that the secretion of HCl was increased to acidify the GIT, consequently making environment to be less ideal for acid intolerant pathogens.

Jimenez-Moreno *et al.* (2009b) observed that the addition of 3% oat hulls or 3% sugar beet pulp in broiler diets was shown to significantly improve the BW and FCR compared to the broilers that were fed control diets. However, the effect of oat hulls on growth rate was more pronounced compared to sugar beet pulp. The beneficial effects of moderate dietary fibre levels in broiler diets might be associated with improved nutrient digestibility (Jimenez-Moreno *et al.*, 2010; Jimenez-Moreno *et al.*, 2009b).

Table 2. 2 Effect of dietary fibre on body weight gain (BWG), feed conversion ratio (FCR) and average daily feed intake (ADFI) in broilers

Item	Age (d)	BWG (g/d)	FCR	ADFI (g/d)	Reference
Control	0-21	31.7 ^b	1.37 ^a	43.2	Gonzalez-Alvarado <i>et al.</i> , 2007
Oat hulls		33.4 ^a	1.33 ^b	44.3	
Soy hulls		33.4 ^a	1.34 ^b	44.6	
Control	0-21	31.2 ^b	1.38 ^a	43.0	Jimenez-Moreno <i>et al.</i> , 2009b
Oat hulls		33.1 ^a	1.30 ^b	43.2	
SBP ¹		32.5 ^{ab}	1.32 ^b	43.9	

¹ SBP – Sugar beet pulp

^{a,b} numbers on one column with different superscripts are significantly different (P < 0.05)

2.8.1 Effect of fibre on Microbial load

Kalmendal *et al.* (2011) conducted a study to evaluate the effects of high fibre sunflower cake on intestinal microbial load. Broilers were fed one of three trial diets (0, 20 and 30% high fibre sunflower cake) *ad libitum* from 15 to 31 days of age. It was noted that the inclusion of either 20 or 30% of high fibre sunflower cake significantly reduced *Clostridium* spp counts in the jejunum

compared to the control at 31 days of age. It was also observed that there was no significant difference in *Lactobacillus* spp counts between the control and 20% high fibre sunflower cake groups. However, the 30% high fibre sunflower cake group had a significantly lower *Lactobacillus* spp counts compared to other treatment groups. It was also shown that there was no significant dietary treatment effect on *E.coli* counts.

Mateos *et al.*, (2012) evaluated the effects of different fibre sources on microbial composition in the GIT of broiler chickens and found that the inclusion of fibre sources in broiler diets had significant influence on the microbial composition in the GIT (Table 2.3). Broilers were fed one of three dietary treatments: (i) control diet, (ii) control diet supplemented with oat hulls at 5% and (iii) control diet supplemented with sugar beet pulp (SBP) at 5%. It was noted that the addition of SBP at 5% in broiler diets significantly increased *Lactobacillus* spp. counts in the crop compared to the addition of 5% oat hulls or control. However, the *Lactobacillus* population in the ceca was not influenced by dietary treatments. It was also shown that the addition oat hulls significantly reduced *C. perfringens* and *Enterobacteriaceae* counts in the ceca compared to the SBP or control group (Mateos *et al.*, 2012). This reveals that the inclusion of insoluble fibre such as oat hulls might reduce the incidence of enteric disorders by inhibiting the growth of pathogens. The inhibitory effect of insoluble fibre on the growth of *C. perfringens* could be attributed to decreased pH of the gizzard and caecal contents (Gonzalez-Alvarado *et al.*, 2007; Mateos *et al.*, 2012).

Table 2. 3 Effect of fibre on Microbial profile (log₁₀ CFU/g) in broilers at 35 days of age (Mateos *et al.*, 2012)

Item	Control	Oat hulls, 5%	Sugar beet pulp, 5%
Crop			
<i>Lactobacillus</i> spp.	7.90 ^b	7.10 ^b	8.40 ^a
Ceca			
<i>Lactobacillus</i> spp.	9.80	8.60	10.0
<i>Clostridium perfringens</i>	5.90 ^a	1.20 ^b	6.20 ^a
<i>Enterobacteriaceae</i>	8.40 ^a	5.90 ^b	8.40 ^a

^{a,b} numbers with different superscripts are significantly different (P < 0.05)

2.9 Medium chain fatty acids

Medium chain fatty acids have a chain length of 6, 8, 10 or 12 carbon atoms, namely, caproic (C6), caprylic (C8), capric (C10) and lauric acid (C12). Medium chain fatty acids (MCFA) have been reported to have high activity against *C. perfringens* (Timbermont *et al.*, 2010) and gram-negative bacteria such as *Salmonella enteritidis* (van Immerseel *et al.*, 2004), *Campylobacter jejuni* (van Gerwe *et al.*, 2010) and *Escherichia coli* (Skrivanova *et al.*, 2006). Moreover, MCFA have been revealed as a good alternative to AGPs in piglets, due to its high antibacterial activity (Dierick *et al.*, 2002).

There are many studies that have been conducted in order to determine the effect of MCFA on broiler performance (Table 2.4). Isaac *et al.* (2013) evaluated the effect of supplementing Aromabiotic Poultry (balanced mixture of medium chain fatty acids, consisting of 60% (C6, C8, C10 and C12) in broiler diets. The addition of 1.2 g/kg of Aromabiotic resulted in a significantly higher ADG compared to the control group, however, no significant differences were observed on FCR, FI or mortality between the treatments. In contrast, Khosravinia, (2015) found no significant

difference on ADG when broiler diets were supplemented with 2 g/kg of Aromabiotic. It was noted that the mortality was significantly lower in broilers that consumed diets containing Aromabiotic compared to those that were fed the control diet. It was also observed that the inclusion of Aromabiotic at 2 g/kg had no significant effect on FI or FCR compared to the control group.

van der Hoeven- Hangoor *et al.* (2013) determined the effects of MCFA (0.3% capric acid and 2.7% lauric acid) on performance parameters of broilers. Broilers on MCFA exhibited a significant better FCR (12 points) and lower FI compared to the broilers that were fed control diets. It was also shown that the BW was not affected by addition of MCFA. Shokrollahia *et al.* (2014) conducted a study to determine the dose response effect of MCFA (C6-C12) on broiler performance. Broilers were fed one of four feed treatments (control, 0.1%, 0.2% and 0.3% MCFA). It was shown that dietary treatments had no significant effect on BW, FCR and FI. Wang *et al.* (2015) evaluated the effect of replacing soybean oil with coconut oil (source of MCFA with a chain length of 6-12 carbon atoms) at 25, 50, 75 and 100%. It was observed that there were no significant differences on ADG, FCR or FI between the treatments.

The improved broiler performance when MCFA were incorporated into broiler diets could be ascribed to healthier gut and high availability of nutrients for absorption. In addition, MCFA inhibits the growth of harmful bacteria in the GIT to reduce competition for nutrients between the pathogens and host, hence, more nutrients will be available for absorption by the host. The lower mortality in birds supplemented with MCFA could be attributed to improved immune function (Khosravinia, 2015).

Table 2. 4 The effect of medium chain fatty acids on average daily gain (ADG), feed conversion ratio (FCR), feed intake (FI) and mortality in broilers

Item	Age (days)	ADG (g/d/bird)	FCR (g/g)	FI(g/d)	Mortality (%)	Reference
Control	0-39	62.6 ^a	1.57 ^a	98.4 ^a	3.80 ^a	Isaac <i>et al.</i> , 2013
MCFA		64.6 ^b	1.56 ^a	101 ^a	3.30 ^a	
Control	0-49	46.0 ^a	1.81 ^a	83.1 ^a	2.70 ^a	Khosravinia, 2015
MCFA		46.4 ^a	1.79 ^a	83.1 ^a	2.10 ^b	
Control	0-34	57.6	1.52 ^a	88.3 ^a	-	van der Hoeven- Hangoor <i>et al.</i> , 2013
MCFA		56.7	1.40 ^b	80.1 ^b	-	
Control	0-42	62.5 ^a	1.85 ^a	116 ^a	-	Shokrollahia <i>et al.</i> , 2014
MCFA		62.1 ^a	1.86 ^a	116 ^a	-	
Control	0-42	58.1 ^a	1.60 ^a	92.9 ^a	-	Wang <i>et al.</i> , 2015
MCFA		57.0 ^a	1.60 ^a	91.1 ^a	-	

^{a,b} numbers on one column with different superscripts are significantly different (P < 0.05)

2.10 Common pathogenic bacteria found in broilers

2.10. 1 *Clostridium perfringens*.

Clostridium perfringens is a gram-positive bacteria, usually found in the GIT of animals and human beings and also in the environment (Songer, 1996). *Clostridium perfringens* strains are divided into five types (A to E) on the basis of production of the four major toxins (α , β , ϵ and ι) (Songer, 1996). The colonisation of broilers by *C. perfringens* might occur in the early stages of

the grow-out period as it can be transmitted from the hatchery, taken up from contaminated feed and water (Craven *et al.*, 2015). *Clostridium perfringens* type A which produces α toxin is mostly found in the GIT of broilers and it causes necrotic enteritis (NE) in broilers chickens (Lensing *et al.*, 2010). Necrotic enteritis is an intestinal disease of high economic impact which appears as either subclinical or as an acute clinical disease (Tsiouris *et al.*, 2015). The incidence of subclinical NE has a negative economic effect as it is estimated to reduce the BW by up to 12% and increase FCR by 10.9% when compared to healthy broilers (Skinner *et al.*, 2010). The signs of clinical NE include high mortality rates, reduced feed intake, diarrhoea and dehydration (Park *et al.*, 2015).

Antibiotic growth promoters have been reported to effectively control and prevent *C. perfringens* in broilers, but AGPs will be banned in the near future (Al-Sagan and Abudabos, 2017). A wealth of research has been conducted to find alternative strategies to prevent and control *C. perfringens* infections in broilers. The use of lauric acid (Timbermont *et al.*, 2010), essential oils (Mitsch *et al.*, 2004), probiotics (Al-Sagan and Abudabos, 2017), prebiotics (Kim *et al.*, 2011) and also vaccination against *C. perfringens* (Schoepe *et al.*, 2001) have been reported to control and prevent *C. perfringens* infections in broilers.

2. 10. 2 Salmonella

Salmonella are gram-negative bacteria that are found in the intestine of broilers and are regarded as foodborne pathogen that causes infection in humans that consume poultry products from infected broilers (Venkitanarayanan *et al.*, 2013). Contaminated chicks, feed, water and dust are among the sources of *Salmonella* infection in broilers (Sasipreeyajan *et al.*, 1996). Poor quality of

litter due to excessive moisture content creates favourable conditions for *Salmonella* to grow and multiply in numbers (Marin and Lainez, 2009).

Salmonella colonisation of the GIT has been reported to decrease the BW (Marcq *et al.*, 2011) and increase FCR in broilers (Vendeplas *et al.*, 2009). The decreased broiler performance due to *Salmonella* infection could be ascribed to strong inflammatory response (Kaiser *et al.*, 2000) that results in reduced digestion and absorption of nutrients and also increased competition for nutrients between the host and *Salmonella* (Vendeplas *et al.*, 2009).

Reducing humidity through adequate ventilation inside the broiler houses is one of the strategies that can be used to control *Salmonella* colonisation in broilers (Bodi *et al.*, 2013). Organic acids, probiotics, prebiotics have been reported to decrease the population of *Salmonella* in the intestine of broilers (Gunal *et al.*, 2006). Medium chain fatty acids, particularly caprylic acid has been reported to reduce *Salmonella* colonisation in the GIT of broilers (Skrivanova *et al.*, 2006; Kollanoor-Johny *et al.*, 2012).

2.11 Conclusion

The increasing consumer and legislation pressure to phase out the AGPs in broiler industry worldwide means that the industry must learn to master the new set of tools to maintain broiler performance and competitiveness without AGPs. Feed and broiler producers should consider utilising the integrated approach to raise broilers without AGPs that combines proper nutrition,

genetics, biosecurity and excellent farm management strategies. The supplementation of feed with innovative additives that are considered to be alternatives to AGPs might also play a crucial role.

There are so many alternatives to AGPs and all have different modes of action. The use of alternatives to AGPs must improve broiler performance at levels comparable to AGPs and must be cost-competitive and effective. The application of the alternatives to AGPs must fit the individual's conditions to ensure the benefit in production. The choice of alternative to AGPs depends on the mode of action, production system and production stage.

Probiotics and pre-biotics products are among the alternatives to AGPs and they have so many different strains available on the market. Some of the strains have the potential while the other strains their effectiveness is not clear. There is therefore a need for further studies to be conducted to describe the mode of action of these strains. Similarly, there are so many sources of insoluble fibre and they all have different physiological factors. Therefore, further research is required to find their minimum and maximum inclusion levels in order to optimize the broiler performance.

CHAPTER 3

EFFECTS OF ESSENTIAL OIL OR ANTIBIOTIC GROWTH PROMOTER ON BROILER PERFORMANCE AND CAECAL *CLOSTRIDIUM PERFRINGENS* COUNTS

3.1 Introduction

The use of antibiotic growth promoters in broiler diets has been reported to have significant effect on performance of broilers particularly when the birds are reared under stressful conditions (Coates *et al.*, 1963). Diseases, poor management and pathogens such *C. perfringens* are some of the factors that may cause stress in broiler chickens. The search for effective alternatives to AGPs is becoming more important as some European countries have banned the use of antibiotic growth promoters due to possible risk of developing bacterial resistance to antibiotics in humans (Elnasri *et al.*, 2014). The restriction in use of antibiotic growth promoters in broiler diets has increased the prevalence rate of economically important diseases such as necrotic enteritis (van Immerseel *et al.*, 2009). Several EO derived from herbs and spices are among the candidates that can be used as an alternative to AGPs due to their antimicrobial activity (Ciftci *et al.*, 2009). It has been reported that EO improves the performance of broilers and also inhibit the growth of *C. perfringens* (Mitsch *et al.*, 2004; Timbermont *et al.*, 2010).

The objective of this study was to evaluate the effects of supplementing the broiler diets with Oligo Essential (essential oil) or AGPs on growth performance and *C. perfringens* counts in the ceca of broiler chickens.

3.2 Materials and Methods

3.2.1 Birds and Housing

The trial was conducted in one of the commercial broiler farms at Rainbow Farms (Ltd), KwaZulu-Natal in South Africa. The average annual minimum and maximum temperature is 13.25 and 27.92, respectively.

A total of ten broiler houses were used for the trial. The houses (80m x 19m) had solid wall with tunnel ventilation. Each house was equipped with nipple drinkers and the feed was supplied via automatic feeder pans. The floor was covered with shavings. The temperature and ventilation were automatically controlled by a control computer box, the temperature was gradually reduced during the grow-out period from 33°C at placement to 20°C at the end of the experiment. Five houses were designated for broilers receiving a control feed and other five houses for broilers receiving feed supplemented with essential oil. The houses were used as experimental units. The control houses (1, 2, 3, 4, 5) were paired with trial houses (7, 8, 9, 10, 11). The houses were paired as follows: 1&7, 2&8, 3&9, 4&10 and 5&11. The chicks placed in paired houses had the same parent flock age, were placed on the same day and also slaughtered at the same age. A total number of 30 000 of day old broiler chicks (Cobb 500) of mixed sex were placed in each house. Feed and water were provided *ad libitum*. The stocking density per house was 22.10 birds/m². The birds were vaccinated against Newcastle disease and Infectious Bursal disease at day-old. The lighting programme is provided in Table 3.1.

Table 3. 1 *Lighting programme*

Age (d)	Light: Dark (hours)
0	24L:0D
1 to 6	23L:1D
7 to 33	16L:8D

3.2.2 Experimental design

There were two treatments for the trial and each treatment group had five replicates. The trial period was 33 days.

3.2.3 Experimental diets

The experiment consisted of two different dietary treatments. Birds in all treatment groups were given a formulated five phase ration consisting of a starter, grower 1, grower 2, finisher and post-finisher, which were mainly based on maize and soybean meal. The starter phase was provided as crumbles and the other four phases were provided as pellets. There were two dietary treatments: (a) Positive control which contained AGPs (Zinc Bacitracin from Ceva); (b) Oligo essential (Castor oil and Cashew nut shell liquid) which is an essential oil was supplemented at 150g/ton (no AGPs added). The diets were mainly based on maize and soybean meal and were formulated using the specification of Rainbow Chicken Ltd. The composition of the control diet is shown in Table 3.2.

Table 3. 2 *The ingredient composition and nutrient content of the control diet, as-fed basis (%)*

Item	Starter	Grower 1	Grower 2	Finisher	Post-Finisher
Maize	55.9	59.1	60.0	62.1	63.7
Soyabean meal (46%+)	36.8	30.3	25.0	22.4	20.5
Sunflower Oilcake	-	-	4.00	4.00	4.00
Poultry By-product	-	5.00	5.00	6.00	6.00
Soya Oil	3.15	1.91	2.64	2.60	2.96
Limestone	1.82	1.65	1.47	1.29	1.30
Monocalcium Phosphate	0.87	0.66	0.46	0.26	0.29
Salt	0.47	0.39	0.40	0.39	0.39
DL-Methionine	0.30	0.28	0.26	0.24	0.22
Valine 20% dilution	0.03	-	-	-	-
Threonine	0.04	0.04	0.04	0.04	0.03
Biolysine 70%	0.30	0.32	0.40	0.38	0.37
Choline Chloride Liquid 75%	0.07	0.07	0.07	0.07	0.03
Vitamin mineral premix	0.20	0.20	0.20	0.20	0.20
Aviax Plus	0.05	0.05	0.05	0.05	-
Zinc Bacitracin 15%	0.03	0.03	0.03	0.03	-
Analysed composition, %					
Moisture	10.3	10.8	10.1	9.40	12.4
Crude protein	20.4	21.8	20.4	19.8	18.3
Ether extract	4.78	5.39	6.34	7.47	6.26
Calcium	1.02	0.81	0.74	0.76	0.76
Phosphorus	0.85	0.54	0.52	0.45	0.45

The diets for both treatments were analysed for moisture (934.01), crude protein (990.03), ether extract (920.39), phosphorus (968.08) and calcium (968.08) (AOAC, 2000).

3.2.4 Measurements

3.2.4.1 Performance parameters

Birds were weighed at 0, 7, 14, 21, 28 and 33 days of age for the determination of BW. Birds were weighed at three points in the house, that is, the front, the middle and the back using the cages suspended in the middle of the house. A total of 1% of the population per house was weighed each week. The birds were penned by lowering the pen over the birds. Birds were placed into the weighing crate two birds at a time and counted by two people. The weight was only recorded once the manual scale has stabilised. The birds were then released from the crate, the scale reset to zero and the next batch of birds counted and weighed. This procedure was repeated until all penned birds were weighed. The ADG was calculated at 7, 14, 21, 28 and 33 days of age as the BW divided by age. Mortality was recorded on a daily basis. Feed intake per house was calculated at 33 days of age as the difference between the amount of feed offered to the birds and remainder of the feed in the feed tank. The bin stock level was recorded by counting the number of rings visible in the tank. The amount of feed in the tank was calculated from the tank capacity excluding the volume without feed. Feed conversion ratio was also calculated at 33 days of age as a FI to BW ratio.

3.2.4.2 *Clostridium perfringens* evaluation

A total of ten birds per house was sacrificed and culled by cervical dislocation at 9 and 30 days of age. The sampled birds were used to examine the presence of caecal *C. perfringens* counts using the Oxoid method (Timbermont *et al.*, 2010).

3.2.5 Statistical Analysis

The data analyses were conducted using the JMP 13.0.0 of SAS. *Clostridium perfringens* counts were transformed to log₁₀ counts before doing statistical analysis. The data were analysed using factorial analysis of variance (treatment, house). The house effect had no significant effect ($P > 0.05$) on broiler performance. Differences between means were determined by Student's t test at significance level of $P < 0.05$.

3.3 Results

The effects of replacing AGPs with EO on the BW, ADG, FI, FCR and mortality are presented in Table 3.3. Considering the whole 33 day experimental period, the EO group had a significantly poorer FCR (8 points) and higher FI compared to the control group. No significant difference in body weight, ADG and mortality was seen in all treatment groups throughout the trial period. The EO group had a significantly lower FI compared to the control group during the period 15-21 days of age.

Table 3. 3 *The effect of essential oil on body weight (BW), average daily gain (ADG), feed intake (FI), feed conversion ratio (FCR) and mortality in broilers from 0 to 33 days*

Item	Control	Essential Oil	P-value
Whole period (d 0 to 33)			
Initial BW, g	42.9	42.6	NS
Final BW, g	1737	1701	NS
ADG, g	52.3	51.2	NS
FI, g	2628	2708	*
FCR	1.51	1.59	*
Mortality	4.43	3.67	NS
Day 0 to 7			
BW, g	196	198	NS
ADG, g	21.9	22.1	NS
FI, g	148	151	NS
FCR	0.75	0.77	NS
Mortality	0.99	1.07	NS
Day 8 to 14			
BW, g	505	498	NS
ADG, g	44.1	43.0	NS
FI, g	549	530	NS
FCR	1.09	1.06	NS
Mortality	1.77	1.97	NS
Day 15 to 21			
BW, g	986	958	NS
ADG, g	68.7	65.6	NS
FI, g	1221	1175	*
FCR	1.24	1.23	NS
Mortality	2.55	2.59	NS

* = P<0.05; NS = not significant

The effect of EO on the *C. perfringens* testing was conducted when birds were 9 and 30 days of age. There was no significant difference on caecal *C. perfringens* counts between the treatments at 9 and 30 days of age (Table 3.4).

Table 3. 4 *The effect of essential oil on Clostridium perfringens count (CFU per g/ml) at 9 and 30 days*

Treatment	9d	30d	P-value ¹
Control	2.71	2.97	NS
Essential Oil	3.06	3.27	NS

¹NS = not significant

3.4 Discussion

The objective of the trial was to determine the effect of EO as an alternative to AGPs on broiler performance under commercial conditions. Supplementing the broiler diets with EO resulted in a significantly poorer FCR and higher FI compared to the control group from 0 to 35 days of age. However, it was also observed that dietary treatments had no significant impact on BW. The results of the current study are in disagreement with Hernandez *et al.* (2004), who evaluated the effects of supplementing broiler diets with 200 ppm EO (oregano, cinnamon and pepper) or AGPs (10 ppm avilamycin) and found no significant difference on BW, FI or FCR between the treatments at 42 days of age. Altop *et al.*, 2017 conducted a study to investigate the effects of supplementing broiler diets with different inclusion levels of EO (sweet gum leaves) on broiler performance. The broilers were fed one of five feed treatments (negative control, negative control supplemented with AGPs, the other three treatments, negative control was supplemented with an EO at 0.04, 0.08 or 0.16 g/kg). It was shown that the broilers that were fed diets supplemented with 0.08 g/kg of EO

had a significantly higher BW, FI and better FCR compared to all other treatments at 42 days of age.

Differences in broiler performance results observed with previously performed studies could be ascribed to differences in composition of EO used, inclusion levels of EO (0.05%, 200ppm or 0.08 g/kg), genetics of the broilers (Ross 308 or Cobb 500), age of the broilers (0-35 or 0-42 d) and rearing conditions (commercial houses which were semi environmentally controlled or fully environmental controlled trail facilities).

In the current study, it was shown that there was no significant effect on caecal *C. perfringens* counts at 9 and 30 days of age. Mitsch *et al.* (2004) reported that supplementing broilers diets with two different blends of EO (blend A: thymol, eugenol, curcumin and piperin and blend B: thymol, carvacrol, eugenol, curcumin and piperin) significantly reduced *C. perfringens* counts in the jejunum and cecum of broilers at 14 and 21 days of age compared to the negative control group.

3.5 Conclusion

The results of the current study showed that the addition of EO (Oligo Essential) into broiler diets resulted in a significantly poorer FCR and higher FI compared to the control group from 0 to 35 days of age. However, it was noted that the BW was not influenced by the dietary treatments. Additionally, there was no significant treatment effect on caecal *C. perfringens* counts at 9 and 30 days of age. It can be concluded that supplementing broiler diets with Oligo Essential did not improve the broiler performance at levels comparable to AGPs in the present study due to its

negative impact on FCR. Further studies at a commercial level are needed to see if the same trend will be observed.

CHAPTER 4

EFFECTS OF PROBIOTIC OR ANTIBIOTIC GROWTH PROMOTER ON BROILER PERFORMANCE

4.1 Introduction

Poultry industry plays a crucial role by positively contributing to economic growth and development in many countries. In commercial farms, broiler chickens are exposed to stressful conditions, some issues are disease related and result in large economic losses (Kabir, 2009). It is therefore very important to control and prevent poultry diseases in order to reduce the risks of major economic losses. Antibiotics have been used for many decades to prevent and treat diseases and also enhance the performance of broiler chickens (Miles *et al.*, 2006). However, the use of AGPs in broiler production has decreased due to concerns raised by consumers about the development of antibiotic resistant bacteria (Dibner and Richards, 2005). This calls for the development of effective replacement products for AGPs.

Probiotics are among the candidates that are considered as replacements for AGPs (Jin *et al.*, 1998). Probiotics are live micro-organisms which, when administered in sufficient amounts might have positive impact on the health of the host as its mode of action include competitive exclusion of harmful bacteria in the GIT (Schneitz *et al.*, 2016). Probiotics can be administered to broilers through feed, water, spray, litter or gavage (Olnood *et al.*, 2015a). Introducing probiotics through spraying to large numbers of chicks under commercial conditions must be efficient and should be administered as early in life as possible as early application of probiotics provides time for

beneficial microbes to colonise the GIT of broiler chickens (Wolfenden *et al.*, 2007). Inconsistent results have been reported concerning the effects of probiotics on broiler performance. It has been reported that the addition of probiotics in broiler diets improve broiler performance (FCR and BW) at levels similar to AGPs (Bai *et al.*, 2013). In contrast, Denli *et al.* (2003) noted that the addition of probiotics in broiler diets significantly reduced BW compared to broilers that were fed diets supplemented with AGPs.

The aim of the trial was to evaluate the effects of *Lactobacillus* based probiotic or AGPs on broiler performance in a commercial farm.

4.2 Materials and Methods

4.2.1 Birds and Housing

The trial was conducted in a commercial broiler farm. A total of six broiler houses were used for the trial. The houses (80m x 19m) had solid wall with tunnel ventilation. Each house was equipped with nipple drinkers and the feed was supplied via automatic feeder pans. The floor was covered with shavings. The temperature and ventilation were automatically controlled by a control computer box, the temperature was gradually reduced during the grow-out period from 33°C at placement to 20°C at the end of the experiment. Three houses were designated for broilers receiving feed supplemented with AGPs and other three houses for broilers receiving feed without AGPs. The day-old chicks from the second treatment were sprayed with *Lactobacillus* based probiotic at the hatchery. Probiotics were administered through the spray cabinet that was triggered every time a crate of chicks was placed inside and the dosing volume was 10ml per 100 chicks.

The probiotics contained a blue dye in order to monitor the administration of probiotics. The houses were used as experimental units. The control houses (1, 2, and 3) were paired with trial houses (4, 5, and 6). The chicks placed in paired houses had the same parent flock age, were placed on the same day and also slaughtered at the same age. The probiotic *Lactobacillus* product is a combination of *Lactobacillus acidophilus*, *Lactobacillus rhamnosus* and *Lactobacillus casei*. A total number of 30 000 of day old broiler chicks (Cobb 500) of mixed sex were placed in each house. The stocking density per house was 20.8 birds/m². Feed and water were provided *ad libitum*. The birds were vaccinated against Newcastle disease and Infectious Bursal disease at day-old. The lighting programme is provided in Table 4.1.

Table 4. 1 *Lighting programme*

Age (d)	Light: Dark (hours)
0	24L: 0D
2 to 4	23L: 1D
3 to 6	22L: 2D
7 to 33	20L: 4D

4.2.2 Experimental diets

Birds in all treatment groups were given a formulated five phase ration consisting of a starter, grower 1, grower 2, finisher and post-finisher. The diets were mainly based on maize and soybean meal and were formulated using the specification of Rainbow Chicken Ltd. The starter phase was provided as crumbles and the other four phases were provided as pellets. The experiment consisted of two different dietary treatments: (i) the control diets contained AGPs (Zinc Bacitracin – Ceva) and anticoccidals, (ii) the trial diets contained only anticoccidals; AGPs were not included in the

diets. The composition of the control diet is shown in Table 4.2. The control diet was analysed for moisture (934.01), crude protein (990.03), ether extract (920.39), phosphorus (968.08) and calcium (968.08) (AOAC, 2000).

Table 4. 2 *The ingredient composition and nutrient content of the control diet, as-fed basis (%)*

Item	Starter	Grower 1	Grower 2	Finisher	Post-Finisher
Maize	55.6	58.6	60.0	62.2	63.4
Soyabean meal (46%+)	36.8	25.7	24.0	22.0	20.9
Sunflower Oilcake	-	4.00	4.00	4.00	4.00
Poultry By-product	-	5.00	5.50	5.50	5.00
Soya Oil	3.47	2.87	3.16	3.35	3.86
Limestone	1.73	1.40	1.23	1.05	1.08
Monocalcium Phosphate	0.90	0.66	0.45	0.26	0.29
Salt	0.45	0.42	0.41	0.41	0.42
Methionine Liquid (Mha)	0.36	0.34	0.34	0.32	0.31
Tryptophan 10% dilution	-	0.13	0.12	0.12	0.11
Threonine	0.06	0.08	0.07	0.08	0.06
Biolysine 70%	0.30	0.45	0.42	0.41	0.39
Choline Chloride Liquid 75%	0.07	0.07	0.07	0.07	0.03
Vitamin mineral premix	0.20	0.20	0.20	0.20	0.20
Monensin	0.05	0.05	0.05	0.05	-
Zinc Bacitracin 15%	0.03	0.03	0.03	0.03	-
Analysed composition, %					
Moisture	10.4	9.50	9.90	10.8	11.6
Crude protein	21.7	20.8	20.3	19.1	17.2
Ether extract	6.23	7.20	7.71	7.39	9.64
Calcium	1.02	0.81	0.74	0.76	0.76
Phosphorus	0.85	0.54	0.52	0.45	0.45

4.2.3 Measurements

4.2.3.1 Performance parameters

Birds were weighed at 0, 7, 14, 21, 28 and 33 days of age for the determination of BW. Birds were weighed at three points in the house, that is, the front, the middle and the back using the cages suspended in the middle of the house. A total of 1% of the population per house was weighed each week. The birds were penned by lowering the pen over the birds. Birds were placed into the weighing crate two birds at a time and counted by two people. The weight was only recorded once the manual scale has stabilised. The birds were then released from the crate, the scale reset to zero and the next batch of birds counted and weighed. This procedure was repeated until all penned birds were weighed. The ADG was calculated at 7, 14, 21, 28 and 33 days of age as the BW divided by age. Mortality was recorded on a daily basis. Feed intake per house was calculated at 33 days of age as the difference between the amount of feed offered to the birds and remainder of the feed in the feed tank. The bin stock level was recorded by counting the number of rings visible in the tank. The amount of feed in the tank was calculated from the tank capacity excluding the volume without feed. The FCR was also calculated at 33 days of age as a FI to BW ratio.

4.2.4 Statistical analysis

The data analyses were conducted using the JMP 13.0.0 of SAS. The data were analysed using factorial analysis of variance (treatment, house). The house effect had no significant effect ($P > 0.05$) on performance parameters. Differences between means were determined by Student's t test at significance level of $P < 0.05$.

4.3 Results

4.3.1 Growth performance

The effects of replacing AGPs with probiotic on the BW, ADG and mortality are presented in Table 4.3. There was no significant difference in BW, ADG, FI, FCR and mortality between the treatments throughout the trial period.

Table 4. 3 *The effect of probiotics on body weight (BW), average daily gain (ADG), feed intake (FI), feed conversion ratio (FCR) and mortality in broilers from 0 to 33 days*

Item	Control	Probiotic	RMSE	P-value ¹
Whole period (d 0 to 33)				
Final BW, g	1568	1607	41.0	NS
ADG, g	47.5	48.7	1.63	NS
FI , g/b	2520	2511	66.8	NS
FCR	1.61	1.56	0.07	NS
Mortality	5.61	4.94	0.40	NS
Day 0 to 7				
BW, g	176	174	8.08	NS
ADG, g	25.2	24.8	1.15	NS
Mortality	1.85	1.31	0.65	NS
Day 8 to 14				
BW, g	481	492	37.9	NS
ADG, g	43.6	45.6	6.30	NS
Mortality	3.13	2.52	0.72	NS
Day 15 to 21				
BW, g	822	813	82.4	NS
ADG, g	48.6	45.8	6.62	NS
Mortality	3.96	3.36	0.72	NS
Day 22 to 28				
BW, g	1301	1293	57.2	NS
ADG, g	68.5	68.5	5.24	NS
Mortality	4.63	3.99	0.69	NS

¹NS = not significant

4.4 Discussion

The aim of the trial was to evaluate the effects of probiotics on broiler performance in a commercial environment. Probiotics had no significant effect on BW of broilers compared to a control from 0 to 33 days of age. The results of the present study are in accordance with Nunes *et al.* (2012), who evaluated the effect of probiotic (*Lactobacillus acidophilus*, *Enterococcus faecium* and *Bifidobacterium bifidum*) and AGPs in broiler diets and found no significant difference on final BW between the treatments at 42 days of age. Similar results were reported by Correa *et al.* (2003), who evaluated the use of two different probiotic products (Calsporin 10 - *Bacillus subtilis* and Estibion - *Lactobacillus acidophilus*, *Lactobacillus casei*, *Streptococcus salivarium*, *Streptococcus faecium*, *Bacillus subtilis*, *Bacillus toyoi* and *Saccharomyces cerevisiae*) and AGPs in broiler diets from 0 to 42 days of age and found no significant treatment effect on BW. Likewise, Yeo and Kim (1997) found no significant difference in BW between broilers that were fed diets supplemented with either probiotic (*Lactobacillus casei*) or AGPs at 42 days of age. In contrast, Denli *et al.* (2003) reported that the addition of probiotics (*Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Bifidobacterium bifidum* and *Enterococcus faecium*) in broiler diets resulted in a significantly lower BW when compared to broilers that received diets supplemented with AGPs at 42 days of age.

In the current study, probiotics had no significant effect on FCR compared to a control from 0 to 33 days of age. These results are in agreement with Gunal *et al.* (2006) who conducted a study to evaluate the effects of probiotic mixture (protexin - *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Bifidobacterium bifidum* and *Enterococcus faecium*) or AGPs in broiler diets from 0 to 42 days of age and found no significant treatment effect on FCR. In contrast, probiotic

Lactobacillus plantarum) inclusion in water showed a significant improvement in FCR compared to broilers that were fed diets supplemented with AGPs at 42 days of age (Gao *et al.*, 2017).

The differences in broiler performance between the current study and previously published studies could be attributed to differences in the strains of probiotics used as a supplement. In the current study the probiotics were applied once at day old chicks through the spray and most past published studies add probiotics in broiler diets or water and this difference in application could be the reason for differences in broiler performance.

4.5 Conclusion

The present study showed that spraying day old chicks with a *Lactobacillus* based probiotic at the hatchery had no significant effect on FCR, FI, BW or mortality at 33 days of age when compared to broilers that were fed diets containing AGPs. The results from this study suggest that *Lactobacillus* based probiotic may be a potential alternative to AGPs as the probiotics has shown to improve broiler performance at levels similar to AGPs. However, more research is required to determine the effect of *Lactobacillus* based probiotic on microbial load.

CHAPTER 5

EFFECTS OF OPTIGUT, PALM KERNEL FATTY ACID DISTILLATE, SUNFLOWER WHOLE SEEDS OR ANTIBIOTIC GROWTH PROMOTER ON BROILER PERFORMANCE, ORGAN WEIGHT, DIGESTA pH AND CAECAL MICROBIAL PROFILE

5.1 Introduction

The use of AGPs in poultry production is under tremendous pressure due to consumer concerns that the continuous exposure to AGPs results to health issues in humans (Kelly *et al.*, 2004). This has forced researchers, nutritionists and broiler production farmers to search for alternatives to AGPs (Rezaei *et al.*, 2011). Butyric acid, medium chain fatty acids and inclusion of low levels of insoluble fibre are among the candidates that are considered as replacements for AGPs.

Butyric acid has pH reducing properties which makes the GIT environment less favourable for harmful bacteria (Panda *et al.*, 2009). This results in reduced microbial population and also decrease competition between the host and microbes for nutrients (Adil *et al.*, 2011). In addition, the use of BA has been reported to improve BW and FCR at levels similar to AGPs (Leeson *et al.*, 2005). Medium chain fatty acids have been reported to have antibacterial properties (Derrick *et al.*, 2002). Furthermore, it has been observed that the addition of MCFA in broiler diets significantly improves broiler performance (Isaac *et al.*, 2013). The use of raw materials that have high insoluble fibre content improves the gizzard development and functionality (Jimenez-Moreno *et al.*, 2010). This stimulate HCL production to acidify the GIT and also reduce the population of acid intolerant bacteria (Mateos *et al.*, 2012).

The aim of the trial was to investigate the effects of Optigut, Palm kernel fatty acid distillate and Sunflower whole seeds on broiler performance, organs weights, intestinal length, digesta pH and caecal microbial profile.

5.2 Materials and Methods

5.2.1 Birds and housing

The experiment was conducted at Ukulinga Research Farm, University of KwaZulu-Natal, Pietermaritzburg, South Africa. The farm is in the subtropical hinterland, located at 30° 24'S, 29°24'E and sea levels are above 700m. The average annual minimum and maximum temperature is 8.98 °C and 25.7 °C, respectively.

Three thousand, three hundred and sixty day-old Cobb 500 male broiler chicks were obtained from a commercial hatchery. Chicks were randomly assigned into six treatment groups of 70 chicks per pen, with eight replicate pens per treatment. Birds in all treatment groups were placed in pens with shavings as a bedding material and were kept in the same experimental house. The stocking density for all treatment groups was 20.6 birds/m² (following commercial standards). Feed and water were provided *ad libitum*. All chicks were vaccinated at the hatchery for Newcastle Disease and Infectious Bursal Disease at day-old. The experimental house had a good lighting and ventilation systems to control temperature, relative humidity and lighting. The lighting programme is provided in Table 5.1. The experimental procedures were approved by the Animal Research Ethics Committee, University of KwaZulu-Natal, South Africa (reference number: AREC/102/015).

Table 5. 1 *Lighting programme*

Age (d)	Light: Dark (hours)
0	23L: 0D
1 to 6	23L: 1D
7 to 35	16L: 8D

5.2.2 *Experimental Diets*

The experiment consisted of six different dietary treatments (Table 5.2). The Surmax (ElancoTM; Avilamycin) was added at 0.04% in treatment 2. The PKFAD (FR Waring (Pty) Ltd) is a source of medium chain fatty acids (C6 - C12) which was included at 2.5% in treatment 3 and 6. The Optigut (ProvironTM; mixture of butyrate and monolaurate) was added at 0.4% in treatment 4. Birds in all treatment groups were given a formulated three phase ration consisting of a starter (0 – 11 d), grower (12-22d) and finisher (23 – 35 d). The starter phase was provided as crumbles and the other two phases were provided as pellets.

Table 5. 2 *The inclusion level of palm kernel fatty acid distillate (PKFAD), Optigut and sunflower whole seeds in dietary treatments (%)*

Treatments	Inclusion level (%)		
	Stater	Grower	Finisher
1. Negative control	No additives	No additives	No additives
2. Negative control supplemented with AGPs (Surmax)	0.04	0.04	0.04
3. Negative control supplemented with PKFAD ¹	2.50	2.50	2.50
4. Negative control supplemented with Optigut ²	0.40	0.40	0.40
5. Negative control supplemented with Sunflower whole seeds ³	4.00	4.00	4.00
6. Negative control supplemented with PKFAD and Sunflower whole seeds	PKFAD at 2.50 and Sunflower whole seeds at 4.00	PKFAD at 2.50 and Sunflower whole seeds at 4.00	PKFAD at 2.50 and Sunflower whole seeds at 4.00

¹PKFAD - source of medium chain fatty acids (C6 - C12); ²Optigut - mixture of butyrate and monolaurate; ³Sunflower whole seeds - source of insoluble fibre

The diets were mainly based on maize and soybean meal and were formulated using the specification of Rainbow Chicken Ltd. The six diets were formulated to contain similar content of metabolisable energy and amino acid profile. The raw materials and calculated nutrient levels of the basal diet are shown in Table 5.3.

Table 5.3 *The ingredient composition and nutrient content of the basal diet, as-fed basis (%)*

Item	Starter	Grower	Finisher
Maize	51.4	55.5	56.2
Soyabean meal (46%+)	22.1	20.2	15.1
Full fat soya	17.2	15.6	20.0
Sunflower Oilcake	4.00	4.00	4.00
Soya Oil	2.00	2.00	2.00
Limestone	1.56	1.31	1.38
Monocalcium Phosphate	0.47	0.16	-
Salt	0.39	0.40	0.42
Methionine DL Powder	0.27	0.26	0.27
Lysine HCL	0.24	0.24	0.24
Threonine	0.05	0.06	0.05
Choline Chloride Liquid 75%	0.07	0.07	0.07
Vitamin mineral premix	0.20	0.20	0.20
Aviax plus	0.05	0.05	0.05
Calculated analysis, %			
Moisture	11.8	11.1	11.9
Crude protein	22.2	21.0	20.3
Ether extract	7.20	7.00	7.70
Calcium	0.74	0.59	0.59
Phosphorus	0.55	0.47	0.43

5.2.3 Experimental Design

Treatments were organised in a randomized block design with six different treatment groups. Each treatment group had eight replicates, with 70 chicks for each replicate. The experimental period was 35 days.

5.2.4 Measurements

5.2.4.1 Performance parameters

Birds were weighed by pen at 0, 7, 14, 21, 28 and 35 days of age for the determination of body weight. The ADG was calculated at 7, 14, 21, 28 and 35 days of age. Mortality was recorded on a daily basis. Feed intake per pen was calculated at 7, 14, 21, 28 and 35 days of age as the difference between the amount of feed consumed by the birds and remainder of the feed in the feeder pans. The FCR was also calculated at 7, 14, 21, 28 and 35 days of age as a FI to BW ratio.

5.2.4.2 Gut health measurements

One bird per pen with BW closest to the average weight of the pen were sacrificed by cervical dislocation at 28 days of age. The selected birds were used to measure/examine the following parameters:

- a) **Lesion scoring:** The intestines were scored for necrotic enteritis following a 0-5 scoring system, 0 being a normal gastrointestinal tract and 5 being the most severe necrotic enteritis (Teirlynck *et al.*, 2011).
- b) **Digesta pH:** The digesta of the cecum, ileum, jejunum and duodenum was collected after the birds were culled. The digesta from each intestinal segment was collected in separate tubes per bird. A digital pH meter was used to measure the pH of the digesta from the cecum, ileum, jejunum and duodenum per bird (Houshmand *et al.*, 2011).

- c) **Microbial Profile:** Caecal contents were cultured anaerobically for examination of Gram-negative (*Campylobacter jejuni*, *Salmonella Enteritidis*) and Gram-positive (*Clostridium perfringens*) bacteria counts (van Immerseel *et al.*, 2004)
- d) **Digestive tract measurements:** After killing the broilers, the gizzard, proventriculus and intestinal segments which include duodenum (from the gizzard outlet to the end of the pancreatic loop), jejunum (segment between the pancreatic loop and Meckel's diverticulum), ileum (segment between Meckel's diverticulum and the caecal junction) and cecum were removed. The empty gizzard and proventriculus were weighed using a digital scale and the length of the intestine segments were measured using a flexible tape (Rezaei *et al.*, 2011).

5.2.4.3 Statistical analysis

The data analyses were conducted using the JMP 13.0.0 of SAS. Bacterial counts were transformed to log₁₀ counts before performing the statistical analysis. The data were analysed as a randomized block design by using an analysis of variance. Differences between means were determined by Turkey's test at significance level of $P < 0.05$.

5.3 Results

5.3.1 Growth performance

The initial BW of chicks was not significantly ($P > 0.05$) different between the dietary treatments (Table 5.4). At the end of the experiment (0 to 35 d), there was no significant ($P > 0.05$) difference in BW and ADG between the treatments. The birds that were fed Sunflower whole seeds had a significantly ($P < 0.05$) lower BW and ADG compared to the other treatments during the starter

period (0 to 7 d). The BW and ADG for PKFAD, PKFAD + Sunflower whole seeds groups were significantly ($P < 0.05$) higher compared to other treatments. During the period of 8 to 14 days of age, PKFAD and PKFAD + Sunflower whole seeds groups had a significantly higher BW while Sunflower whole seeds group had a significantly ($P < 0.05$) lower BW compared to other treatments. However, there was no significant ($P > 0.05$) difference in ADG between the treatments for the period of 8 to 14 days of age. There was no significant ($P > 0.05$) treatment effect on BW and ADG during the period of 15 to 35 days of age. In addition, it was observed that there was no significant ($P > 0.05$) difference in FI between the treatments during the overall experimental period (0 to 35 d).

5.3.2 Feed conversion ratio

There was no significant ($P > 0.05$) difference on FCR between the treatments during the overall experimental period (0 to 35 d). At 7 days of age, the negative control and PKFAD + Sunflower whole seeds groups had a significantly ($P < 0.05$) poorer and better FCR compared to other treatments, respectively. A significant treatment effect was observed between the treatments at 0-14 days of age. The negative control and PKFAD + Sunflower whole seeds groups had a significantly ($P < 0.05$) poorer and better FCR compared to other treatments, respectively. The Optigut group had a significantly ($P < 0.05$) better FCR compared to other treatments at 0-21 days of age. There was no significant ($P > 0.05$) treatment effect on FCR between the treatments at 0-28 days of age.

5.3.3 Mortality

There was no significant ($P > 0.05$) treatment effect on mortality between the treatments throughout the experimental period.

Table 5. 4 The effect of antibiotic growth promoter, Optigut, palm kernel fatty acid distillate and sunflower whole seeds on body weight (BW), average daily gain (ADG), feed intake (FI), feed conversion ratio (FCR) and mortality in broilers from 0 to 35 days.

Item	Neg. Control ¹	Pos. Control ²	PKFAD ³	Optigut	SW ⁴	PKFAD + SW ⁵	RMSE	P-value
Whole period (0 to 35d)								
Final BW, g	2219	2202	2244	2223	2196	2234	23.7	NS
ADG, g	62.2	61.7	62.9	62.3	61.5	62.6	0.68	NS
FCR	1.44	1.45	1.47	1.43	1.45	1.46	0.01	NS
Mortality	8.42	6.94	6.23	7.68	9.46	6.64	0.88	NS
FI, g	3198	3180	3300	3180	3185	3255	35.5	NS
Day 0 to 7								
BW, g	177 ^{ab}	179 ^{ab}	183 ^a	178 ^{ab}	175 ^b	183 ^a	1.85	**
ADG, g	19.2 ^{ab}	19.3 ^{ab}	19.9 ^a	19.2 ^{ab}	18.7 ^b	20.0 ^a	0.25	**
FCR	0.85 ^a	0.82 ^{ab}	0.83 ^{ab}	0.83 ^{ab}	0.83 ^{ab}	0.81 ^b	0.01	*
Mortality	1.44	2.32	2.85	3.76	4.09	2.35	0.86	NS
Day 8 to 14								
BW, g	451 ^{ab}	455 ^{ab}	467 ^a	452 ^{ab}	447 ^b	465 ^a	3.85	**
ADG, g	39.1	39.5	40.5	39.2	38.9	40.3	0.46	NS
FCR	1.11 ^a	1.08 ^{bc}	1.10 ^{ab}	1.08 ^{bc}	1.10 ^{ab}	1.07 ^c	0.01	***
Mortality	3.05	3.74	3.75	4.12	5.52	3.22	0.89	NS
Day 15 to 21								
BW, g	934	938	959	932	922	948.9	8.82	NS
ADG, g	69.0	69.1	70.3	68.6	67.9	69.1	1.05	NS
FCR	1.18 ^{ab}	1.17 ^{ab}	1.18 ^a	1.16 ^b	1.18 ^{ab}	1.17 ^{ab}	0.01	*
Mortality	3.94	4.09	4.10	4.83	5.70	4.12	0.87	NS
Day 22 to 28								
BW, g	1605	1596	1635	1588	1575	1616	16.07	NS
ADG, g	95.8	94.0	96.6	93.6	93.2	95.3	1.32	NS
FCR	1.31	1.32	1.31	1.31	1.33	1.31	0.01	NS
Mortality	8.42	6.94	6.23	7.68	9.46	6.64	0.88	NS.

¹Negative control; ²Positive control; ³Palm kernel fatty acid distillate; ⁴Sunflower Whole seeds; ⁵Palm kernel fatty acid distillate + Sunflower Whole seeds. ^{a, b, c} Means within a row with unlike superscripts are significantly different (P<0.05). * = P<0.05; ** = P <0.01; ***P<0.00; NS = not significant

5.3.4 Microbial profile

The results of caecal microbiota (Table 5.5) indicate that there was no significant treatment effect for *C. perfringens* and *Campylobacter*. All samples were negative for *Salmonella* in all dietary treatments.

Table 5. 5 The effect of antibiotic growth promoter, Optigut, palm kernel fatty acid distillate and sunflower whole seeds on caecal log₁₀ bacterial (*C. perfringen*, *Campylobacter jejuni* and *Salmonella Enteritidis*) counts in broilers at 28 days

Treatment	<i>C. perfringens</i>	<i>Campylobacter jejuni</i>	<i>Salmonella Enteritidis</i>
Negative control	3.00	3.27	Negative
Positive Control	2.24	3.23	Negative
PKFAD ¹	2.67	3.76	Negative
Optigut	3.48	3.64	Negative
SW ²	2.68	3.35	Negative
PKFAD + SW ³	2.56	3.51	Negative
P-Value	NS	NS	

¹Palm kernel fatty acid distillate; ²Sunflower Whole seeds; ³Palm kernel fatty acid distillate + Sunflower Whole seeds.

5.3.5 Intestinal length

The effects of dietary treatments on relative lengths of intestinal segments are presented in Table 5.6. There was no significant ($P > 0.05$) treatment effect on relative length of intestinal segments.

Table 5. 6 *The effect of antibiotic growth promoter, Optigut, palm kernel fatty acid distillate and sunflower whole seeds on relative intestinal length (cm/kg BW) at 28 days*

Treatment	Duodenum	Jejunum	Ileum	Caeca
Negative control	15.5	37.7	38.8	9.43
Positive Control	15.8	38.5	39.8	9.42
PKFAD ¹	14.9	38.5	39.6	9.02
Optigut	15.8	40.0	41.4	9.17
SW ²	15.4	39.8	39.4	9.73
PKFAD + SW ³	15.8	38.8	40.3	9.48
P-Value	NS	NS	NS	NS

¹Palm kernel fatty acid distillate; ²Sunflower Whole seeds; ³Palm kernel fatty acid distillate + Sunflower Whole seeds.

5.3.5 Digesta pH

There was no significant ($P > 0.05$) treatment effect on pH of the gizzard, duodenum, jejunum, ileum and caeca (Table 5.7).

Table 5. 7 *The effect of antibiotic growth promoter, Optigut, palm kernel fatty acid distillate and sunflower whole seeds on digesta pH at 28 days*

Treatment	Gizzard	Duodenum	Jejunum	Ileum	Caeca
Negative control	4.26	6.00	5.88	5.88	6.00
Positive Control	4.25	5.38	5.63	6.00	6.38
PKFAD ¹	4.38	6.00	5.63	5.88	6.00
Optigut	4.13	5.75	5.88	6.00	6.38
SW ²	4.13	5.63	5.75	5.88	6.00
PKFAD + SW ³	4.38	5.38	5.50	5.88	6.13
P-Value	NS	NS	NS	NS	NS

¹Palm kernel fatty acid distillate; ²Sunflower Whole seeds; ³Palm kernel fatty acid distillate + Sunflower Whole seeds.

The gizzard and proventriculus weights are presented in Table 5.8. There was no significant ($P > 0.05$) treatment effect on gizzard and proventriculus weight.

5.3.6 Gizzard and proventriculus weight

Table 5. 8 *The effect of antibiotic growth promoter, Optigut, palm kernel fatty acid distillate and sunflower whole seeds on gizzard and proventriculus weight at 28 days*

Treatment	Gizzard	Proventriculus
Negative control	19.1	3.76
Positive Control	19.0	3.71
PKFAD ¹	19.5	3.82
Optigut	20.1	3.85
SW ²	21.8	4.04
PKFAD + SW ³	20.1	3.80
P-Value	NS	NS

¹Palm kernel fatty acid distillate; ²Sunflower Whole seeds; ³Palm kernel fatty acid distillate + Sunflower Whole seeds

5.3.7 Lesion Scoring

The intestines were scored for necrotic enteritis and there were no birds observed with necrotic enteritis across all the treatment groups.

5.4 Discussion

5.4.1 Growth performance

There was no significant effect of insoluble fibre (sunflower whole seeds groups) on BW, FI, FCR or mortality compared to a positive control from 0 to 35 days of age. The results of the present study are in agreement with Hetland *et al.* (2003) who found no significant effect of oat hulls on BW of broiler chickens.

In contrast, Abazari *et al.* (2016) reported that the broiler diets containing 7.5g/kg rice hulls which is source of insoluble fibre significantly improved the growth rate and FCR of broilers compared to a control. Likewise, Rezaei *et al.* (2011), found that micronized insoluble fibre significantly increased ADG and improved FCR. The positive effects of insoluble fibre on BW and FCR were attributed to improved gizzard activity that acidified the gut and subsequently reduced the proliferation of harmful micro-organisms (Gonzalez-Alvarado *et al.*, 2007; Mateos *et al.*, 2012). Consequently, increasing nutrient availability for absorption due to reduced competition for nutrients between the host and harmful bacteria. The difference in broiler performance between the present study and other previously published studies could be attributed to differences in the composition of insoluble fibre.

The BW of PKFAD group was significantly higher compared to the positive control group during the early stages of growth. The improved BW was associated with higher FI. The significant improvement in BW in the early stages of the PKFAD group could be explained by the antimicrobial properties of MCFA. This implies better nutrient utilisation due to reduced competition between the microbes and the host for nutrients. However, the addition of PKFAD

that is rich in MCFA had no significant effect on BW, FCR or FI from 0 – 35 days of age. These results are similar to those found by Wang *et al.* (2015), where broiler performance parameters were not significantly affected by the use of coconut oil (rich in MCFA) at 42 days of age. van der Hoeven-Hangoor *et al.* (2013) reported that supplementing broiler diets with MCFA (0.3% C10 and 2.7% C12) resulted in a significant improvement in BW and FCR compared to a control group at 34 days of age. The difference in broiler performance between the present study and other studies could be due to differences in composition and sources of MCFA.

There were no significant differences on BW, FI, FCR or mortality between broilers fed Optigut and the positive control from 0 to 35 days of age. The results of the present study are in agreement with Aghazadeh and TahaYazdi, (2012) who found no significant effects of BA supplementation in broiler diets on final BW and FCR in broilers. On the contrary, BA has been reported to significantly improve BW and FCR on broiler chickens (Salmanzadeh, 2013). Similarly, Zeitz *et al.* (2015) found that addition of fat rich in lauric acid had a significant better FCR in broilers compared to a control. However, there is little published literature that has evaluated the combination of butyric and lauric acid on broiler performance.

5.4.2 Gizzard and proventriculus weight

There was no significant treatment effect on the weight of the gizzard and proventriculus. The birds that were fed diets that contained sunflower whole seeds were anticipated to have a significantly heavier gizzard compared to positive control group. The results of the current study are in accordance with Rezaei *et al.* (2011) who reported that the inclusion of micronized insoluble

fibre had no significant effect on the gizzard weight of broilers at 42 days of age. The results of the present study are in disagreement with Hetland *et al.* (2003) who found that the inclusion of oat hulls as a source of insoluble fibre in broiler diets resulted in a significant increase in gizzard weight of broilers at 33 days of age. The beneficial effects of insoluble fibre were attributed to improved gizzard development and functionality in that study.

5.4.3 Gut pH

The pH of different segments of the gastro-intestinal track was not influenced by dietary treatments. High buffering capacity of the gastro-intestinal tract of broilers could be associated with non-significant effects of dietary treatments on pH (Houshmand *et al.*, 2011). The pH of the birds that were fed diets that contained sunflower whole seeds and Optigut were anticipated to have lower pH in the gut compared to a positive control. On the other hand, it has been reported that insoluble fibre (Sacranie *et al.*, 2012) and butyric acid (Panda *et al.*, 2009) have pH reducing properties.

5.4.4 Microbial profile

There was no significant difference in caecal counts for *C. perfringens*, *C. jejuni*, and *Salmonella enteritidis* between the treatments. The lack of response in microbial profile could be due to broilers in the trial being healthy and without environmental challenges (Levy *et al.*, 2015). The dietary treatments in the present study have been reported to have high affinity against pathogens. It has been reported that MCFA display antimicrobial activity against *C. perfringens*, *Salmonella enteritidis* and *C. jejuni* (van Immerseel *et al.*, 2004; Skrivanova *et al.*, 2006; Timbermont *et al.*,

2010; van Gerwe *et al.*, 2010). Insoluble fibre (Mateos *et al.*, 2012) and BA (Fernandez–Rubio *et al.*, 2009) have been reported to have antimicrobial properties.

5.5 Conclusion

The results of the present trial showed that there was no significant treatment effect in broiler performance parameters, organs weights, intestinal length, digesta pH and caecal microbial profile to 35 days of age. The broiler performance of the studied alternatives to AGPs was surprisingly comparable to a negative and positive control groups. This suggest that the trial was conducted in a hygienic facility as it was anticipated that the negative control group would have poorer broiler performance. It was recommended to conduct challenge studies with *C. perfringens* to further investigate the effects of Optigut, Palm kernel fatty acid distillate and Sunflower whole seeds as alternatives to AGPs.

CHAPTER 6

GENERAL CONCLUSIONS

There is an increasing consumer and legislation pressure for the broiler industry to rear broilers without AGPs across the world due to concerns of development of resistance bacteria. This clearly means that the broiler producers must adapt to producing the broilers without AGPs. This study provided further information for several alternatives to AGPs that might be used to replace AGPs. However, the available different alternatives to AGPs have been reported to have inconsistent effect on broiler performance. Furthermore, a combination of strategies such as nutrition, genetics, biosecurity and AGPs replacement products may be used to improve broiler performance at levels comparable to AGPs. The selection of the economically feasible approach is highly dependent on the production system and also on the company's philosophy.

When broilers were fed diets supplemented with EO (Oligo Essential) or AGPs from 0 to 33 days in a commercial farm (Chapter 3), it was noted that the broilers that were fed diets supplemented with Oligo Essential had a significantly poorer FCR and higher FI when compared to broilers that were fed diets containing AGPs. However, no effect of dietary treatment was seen on the BW. In addition, the caecal *C. perfringens* counts at 9 and 30 days of age were unaffected by feed treatment. It can be concluded that supplementing broiler diets with Oligo Essential had negative effects on broiler performance in the present study. Further studies of Oligo Essential on broiler performance might provide an explanation for results in the present study.

The results from the present study suggest that spraying day old chicks with a *Lactobacillus* based probiotic at the hatchery will improve broiler performance at levels comparable to AGPs (Chapter 4). It was observed that the *Lactobacillus* based probiotic had no significant effect on FCR, FI, BW and mortality at 33 days of age when compared to broilers that were fed diets containing AGPs. It can be concluded that *Lactobacillus* based probiotic may be a potential alternative to AGPs as the broiler performance was comparable to broilers that received diets supplemented with AGPs. However, more research is required to determine the effects of *Lactobacillus* based probiotic on microbial load.

It was observed that the use of Optigut, Palm kernel fatty acid distillate or Sunflower whole seeds as replacement for AGPs had no significant effect on broiler performance, organs weights, intestinal length, digesta pH and caecal microbial profile when compared to the positive and negative control (Chapter 5). It can be concluded that the lack of response between the treatment groups could be due to hygienic trial environment. It was therefore recommended to further investigate the effects of these alternatives to AGPs in a challenge study with *C. perfringens*.

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