

**HUMIC ACID AND ENZYMES INCLUSION IN CANOLA-BASED BROILER  
DIETS: EFFECTS ON PHYSIOLOGICAL AND MEAT QUALITY PARAMETERS**

By

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## DECLARATION

I, the undersigned, hereby confirm that the work contained in this research dissertation is my own original work for a Degree of Masters of Science in Agriculture in Animal Science working under the supervision of Professor Upenyu Marume and Professor Victor Mlambo. This dissertation has not been previously submitted to any University. Materials and evident information from any other sources has been fully recognized.

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## GENERAL ABSTRACT

This study was undertaken to investigate the effects of potassium humate and Axtra XAP enzyme (Xylanase + Amylase + Protease) as dietary additives on growth performance, protein utilisation efficiency, blood parameters, meat quality and tibia bone parameters in broilers fed canola-based diets. Two hundred and twenty broiler chickens were randomly allotted to 5 dietary treatments: control (commercial broiler diet); CM (17.5 % canola meal inclusion); CMenz (17.5% CM inclusion + 0.3 g/kg Axtra XAP); CMPh (17.5% CM inclusion + 1.5% Potassium Humate, PH) and CMenzPh (17.5% CM inclusion + 1.5% PH + 0.3 g/kg Axtra XAP). The feeding trial started at the grower phase when the birds were 14 days of age. Intake and weight data were used to calculate average daily feed intake (ADFI), feed conversion ratio (FRC) and average daily gain (ADG). There were no significant ( $P > 0.05$ ) differences on ADFI across all treatments for both grower and finisher phases. However, broilers offered CM had higher ( $P < 0.05$ ) ADG ( $71 \pm 1.08$  g/d) compared to birds on all the other diets. Cumulative weight gain of birds fed diet CMenzPh was the highest throughout the experimental period. Dietary treatment significantly ( $P < 0.05$ ) affected protein utilisation and growth efficiency parameters in both grower and finisher phases apart from the protein consumed (PC) in the finisher phase, specific growth rate was also highest in CM chickens compared to all other treatments. In all instances, the control diet promoted the lowest values for PC, PER, specific growth rate (SGR) and growth efficiency (GE) in the grower phase. Haematological parameters were not influenced ( $P > 0.05$ ) by dietary treatments. The serum biochemistry indices, AST and sodium, were significantly ( $P < 0.05$ ) influenced by dietary treatments but not ALP, ALT, total protein, potassium, albumin, total calcium, cholesterol and magnesium. Diet had no effect on all carcass traits apart from breast weight and breast muscle index of broilers being significantly different. The results on meat quality measurements also showed a lack of significant effect of diet on pH and temperature measurements, drip loss and shear force values of the breast muscle.

However, diet had a significant effect on the 3 meat colour coordinates and water-holding capacity (WHC). With regards to meat colour, broiler muscle in the control and CMPh groups (52.94 and 52.91, respectively) had the highest ( $P < 0.05$ ) values for lightness ( $L^*$ ), whilst the meat from broilers fed CMenzPh had the lowest (47.94). With regards to fatty acid profile, higher values for PUFAs, n-3 fatty acids and n-6 fatty acids were observed in the CM containing diets particularly the CMPh group. The inclusion of CM, enzyme complex and humic acid salt increased the PUFA/SFA ratio whilst at the same time reducing the n-6/n-3 ratios. Diet had an effect on latency to lie test with broilers in CMenz having the highest tendency to lie (2.88 minutes). The highest standing persistency was observed in CMenzPh (11.19 minutes). Diet had no effect on tibia biomechanics. Diet had an influence ( $P < 0.05$ ) on the macro mineral (calcium, phosphorus, magnesium and potassium) content apart from sodium. Intestinal morphometric parameters demonstrated some differences in the height and width of the intestinal villi and in the width of the intestinal crypts. Gross lesions analysis showed high prevalence of rickets in CMenz, whilst the inclusion of canola and PH appeared to improve distribution and density of lymphoid tissue in the peripheral and central follicles building tissues of the bursae of fabricius and thymus. Overall, canola meal was shown to have potential as an alternative of soybean meal in broiler diets. Collectively, the findings from the study can be helpful in designing less-expensive feed formulations, physiological and meat quality in poultry farming systems in future.

Keywords : Growth, broilers, performance, protein, utilization, efficiency, blood, meat, quality, Canola, humic acid, enzyme and supplementation.

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## LIST OF ABBREVIATIONS

a*	Redness
L*	Lightness
b*	Yellowness
ADFI	Average Daily Feed Intake
ADG	Average Daily Gain
BBS	Breaking bone strength
BW	Body Weight
CM	Canola Meal
FAMEs	Fatty Acid Methyl Esters
FAs	Fatty Acids
FCR	Feed Conversion Ratio
GE	Growth efficiency
GLM	General Linear Model
Gls	Glucosinolates
HA	Humic Acid
NWU	North-West University
PC	Protein Consumed
PER	Protein Efficiency Ratio
PH	Potassium Humate
PMM	Pectoralis major muscle
SA	South Africa
SBM	Soybean Meal
SGR	Specific Growth Rate
TBS	Tibia Breaking Strength
TDPE	Tibia Diameter Proximal End
TWD	Tibia Width Diameter
TDDE	Tibia Diameter Distal end
TLD	Tibia Length Diameter
TMC	Tibia Mineral Content
WHC	Water Holding Capacity
XAP	Xylanase Amylase Protease

## CHAPTER 1

### 1. GENERAL INTRODUCTION

#### 1.1 Background

In today's ever-changing poultry industry, good management and feeding practices are essential for sustainability and profitability. Recently, intensive broiler production has achieved remarkable gains in terms of efficient and economical production of safe wholesome and high quality chicken meat (Hashemia *et al.*, 2012). This has been achieved through the use of high value protein sources and feed additives that maximize productivity, such as soybean meal (SBM), enzyme and antibiotic growth promoters. However, high value protein sources such as SBM are becoming expensive due to competition between humans and livestock because SBM serves as food to humans and feed to animals, which increases the demand resulting in high prices on the world market (Shi *et al.*, 2012). Moreover, the use of antibiotics and growth promotants in poultry diets are causing great public concerns due to the associated risks from the accumulation and persistency of exogenous residues in poultry meat products (Engberg *et al.*, 2000; Attia *et al.*, 2011). Among other alternative protein sources, canola meal has been shown to have great potential (Nowlin, 1991).

Canola meal (CM) is a derivative of low-erucic-acid (2%) oilseed rape commonly known as Canola. It is rich in essential minerals and vitamins with comparable amino acid profile to SBM and (Naseem *et al.*, 2006). Nevertheless, the use of canola meal in broiler diets can be limited by the presence of anti-nutritional factors, such as glucosinolates (Gls), phytic acid and tannins (Khajali and Slominski, 2012). However, the current cultivars being grown have been developed to have low erucic-acid and glucosinolate content with little effects on utilization by livestock. Despite its many attributes, canola meal tends to have lower non-starch

polysaccharides (35-39%), lower energy (2,000 kcal/kg AME) and higher dietary fiber (12 %) content compared to soybean meal (Jia *et al.*, 2012; Bell, 1993; Slominski *et al.*, 2012).

Digestibility of low quality protein sources such as canola meal may be improved significantly by use of exogenous enzyme and other natural feed additives such as potassium humate (PH). Exogenous enzymes including proteases, carbohydrases and phytases have been used to increase digestibility of low quality protein feed ingredients ultimately promoting growth in broilers (Preston *et al.*, 2001). Potassium humate is an important humic acid salt, with great potential as a growth promotant in animal feeds (Kocabagli *et al.*, 2002). It is the final product of lignite or leonardite when treated with an alkali and is produced in several ways. The reported important properties of Potassium humate includes among others the anti-bacterial, anti-inflammatory, anti-viral and anti-oedematous effects in animals (Kuhnert *et al.*, 1991). The concept of using natural organic acids such as potassium humate as an alternative feed additive in animal nutrition has provoked increasing interest among researchers, particularly after the ban was instituted on the use of antibiotic in feeds as growth promoters (Ceylan *et al.*, 2003; Karaoglu *et al.*, 2004).

## **1.2 Problem statement**

The use of PH and enzymes to improve digestibility of canola meal in broiler diets has not been widely investigated. Information on effects of inclusion of PH and enzymes on the performance of broilers is scant in literature and requires comprehensive investigation. Critically, there is a need to accumulate information on how the utilisation of poor protein sources can be improved through the interactive effects of enzymes and PH. Such analysis may enable poultry farmers to implement feeding strategies that incorporate the use of the less-expensive canola meal to optimise broiler production.

### **1.3 Justification**

The reason for exploring natural feed additives and exogenous enzymes that can be added to canola is to develop less-expensive diets that can supply all the required nutrients for optimum production of broiler. Generally, in the poultry production industry, there is overdependence on soybean meal as a major protein source. In this case, it is becoming too expensive due to competition between human consumers and livestock animals, hence, resulting in the need to import additional supplies. Studies on the use of enzyme and humate in diets can provide vital information in enhancing flexibility in formulation of low-cost, highly digestible diets. This research can, therefore, provide a better understanding on the influence of exogenous enzyme and potassium humate on growth performance, physiological responses and meat quality of broiler chickens fed canola-based diets.

### **1.4 Objectives**

The main objective of this study was to assess the effect of using potassium humate and Aextra XAP (xylanase, amylase and proteases) as additives in canola-based diets on growth performance, blood parameters, meat quality and tibia bone parameters of broilers.

*The specific objectives of the study are:*

1. To determine the effect of potassium humate and Aextra XAP as dietary additives on growth performance, protein utilisation efficiency and blood parameters of broilers fed canola-based diets.
2. To determine the influence of potassium humate and Aextra XAP on carcass characteristics, the quality and fatty acid profiles of meat from broilers fed canola-based diets.
3. To determine the potassium humate and Aextra XAP effects on tibia bone parameters and incidences of rickets in broilers fed canola-based diets.

## 1.5 Hypothesis

*H<sub>0</sub>*: Inclusion of potassium humate and Aextra XAP as dietary additives has no influence on growth performance, protein utilisation efficiency, blood parameters, carcass characteristics, meat quality, fatty acid profiles, tibia bone parameters and incidences of rickets on broilers fed canola-based diets.

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## CHAPTER 2

### 2. LITERATURE REVIEW

#### 2.1 Introduction

Broiler chickens (*Gallus gallus domesticus*) are gallinaceous domesticated fowls that are bred and raised for meat production, reaching slaughter weight of ~ 2 kg in 35 - 42 days. Poultry meat production involves the use of high performing breeds that offer high quality meat and feed formulations that guarantees optimum performance and health status. Worldwide the poultry industry has been the most dynamic and one of the most actively expanding livestock production sectors for the past two decades. The primary objective in numerous poultry enterprises is meat production and this is being achieved mainly through the use of broilers with high feed conversion efficiency, a rapid growth rate and high processing yield.

#### 2.2 Protein sources for broiler diets

For many years, the major protein source in broiler diets has been soybean meal (SBM). Soybean meal excellent protein ingredients that can effective balance nutrients from grains to produce nutritious feed for optimum poultry performance (Pettersson and Pontoppidan, 2013). It has a balanced amino acid profile that sufficiently complements the amino acid profile of corn forming diets that support optimum and economic performance in broilers. Soybean meal accounts for nearly 69% protein sources used of all animal feeds followed by rapeseed meal worldwide and it is a constant product that the nutritionists can plan on for providing key nutrients significant for least cost computer feed formulation. However, due to competition between humans and livestock for the same protein source, SBM is becoming rather expensive and unaffordable to most poultry producers, bringing about a need to seek alternative protein sources such as canola meal.

The use of canola meal in the poultry industry has been minimal. Canola is a new variety of rapeseed which was developed using plant breeding techniques to reduce the toxic glucosinolate content (*Brassica napus* L.) which is a member of the Brassicaceae family (syn. Cruciferae). In addition, it is the most frequently grown Brassica species in the world having an erucic acid (C22:1) content at the most of 2% by weight and thus the solid component of less than 30 micromoles of the glucosinolates per gram of oil free meal (Jia *et al.*, 2012). Canola meal is known to be a high quality product, however low in protein compared to soybean meal (Spragg *et al.*, 2014). Canadian Canola Council reported that the minimum crude protein assured for Canola meal is 36% (8.5 % moisture basis). Conversely, the actual protein content is commonly about 36–39% depending on the distinction of canola seed composition annually in line with the growing and harvesting circumstances (U *et al.*, 2002; CCC, 2009). It also contains polyphenols (8%), cellulose (4–6%) and non-cellulosic polysaccharides (13–16%) which consist predominantly of pectic substances (Slominski *et al.*, 1990). Due to the considerable protein content in CM it can be a very good alternative vegetable protein source in place of soybean meal in broiler diets.

### **2.3 Canola meal as a source of protein on broilers**

Khajali and Slominski (2012) reported that CM compares well with SBM with regard to amino acid profiles important in broiler diets. Canola meal contains large amounts of methionine and cysteine. Canola meal, however, contains low levels of lysine and arginine (Arg), a consideration that should be made when introducing CM to broiler diets at high inclusion levels. However, CM contains considerable amounts of calcium, iron, manganese, selenium, and many B vitamins (Newkirk, 2009). Meng *et al.*, (2006) observed that inclusion of 150 g/kg of canola meal in a mash diet from day 5 to 18 resulted in lower fat, (less) protein digestibility and negatively affected apparent metabolic energy (ME) that is corrected for nitrogen (AMEn)

of the diet. The report suggests uncertainty in nutrient utilization, and hence complete substitution of SBM with CM may not be practically feasible in the poultry industry. Canola meal as a protein source, also has high fibre levels and anti-nutritional factors capable of hindering the growth of the animal's performance by interfering with absorption of nutrients in the digestive system (Bonnardeaux, 2007). The utilisation of CM can be improved through the use of feed additives such as enzymes, probiotics, and organics acids such as humic acids which may alter digestion dynamics of CM for the benefit of animals.

#### **2.4 Dietary feed additives in poultry diets**

Over the years, the efficiency of the bird's growth, general health status and feed utilization have been achieved through use of various feed additives, including antibiotics, enzymes, probiotics organic acids and others. Additives that are to be included in feed must however, have approval for use and then be used as per stipulation with respect to inclusion rates and feeding durations. They are also specific for the type and age of birds being fed. Antibiotics feed additives act as growth promoters by inhibiting disease existence and the treatment of existing diseases, therefore improving the effectiveness of poultry production. Kalmar *et al.*, (2011) suggested that, enhanced utilization of dietary fat as an energy source is a possible underlying basis. Due to increasing public concerns on the use of antibiotics, alternatives such as organic acids, including humates can play a similar role as the antibiotics. On the other hand, utilization of the poor protein sources such as Canola can be improved through use of the enzymes and organics acids.

#### **2.5 Enzyme influences on broiler performance**

The use of enzymes in diets has provided a very intrinsic to improve digestibility of poor sources of nutrients giving greater flexibility in low cost diets formulation (Zakaria *et al.*,

2012). The variability in reaction and effectiveness of exogenous enzymes depend on the type of feed and nutritional composition of the feed. Depending on the nutritional compositions of the feed resources various enzymes including phytase, xylanase, glucanase, protease, amylase and numerous other enzymes have been included in broiler diets (Ghazi *et al.*, 2002; Yu *et al.*, 2002; Gracia *et al.*, 2003). Canola meal, with a high fibre content and low protein content compared to SBM, requires the use of enzyme complexes including proteases, phytases and carbohydrases which may facilitate optimum utilisation of protein, phosphorus and energy for quality enhancements of Canola meal (Slominski and Campbell, 1990; Simbaya *et al.*, 1996; Kocher *et al.*, 2000). Proteins serve a vital metabolic role as blood plasma proteins, enzymes, hormones and antibodies, each having a specific role in the body (Pond *et al.*, 1995). However, protein is also one of the most expensive ingredients in poultry diets. Soybean protein is favoured due to its well-balanced essential amino acid profile, permitting it to balance most diets (Ravindran, 2013). Recently, more research has focused more on effect of proteases inclusion in broiler diets on the overall production performance, nutrient digestibility and meat quality (Fidelis *et al.* 2010; Angel *et al.* 2011; Frietas *et al.* 2011; Dosković *et al.* 2015).

Enzyme supplementation may possibly reduce the inconsistency in nutritional value between feedstuff and may improve accuracy of the feed formulations. Gracia *et al.* (2003) found that  $\alpha$ -amylase inclusion in maize and SBM diet significantly improved digestibility. Furthermore,  $\alpha$ -amylase could reduce the relative weight of pancreas but with no effects on the gizzard, the liver or the small intestines (SI). Reports on the benefits of enzymes in broilers are inconsistent with some reports indicating that benefits are realised more in the grower or finishing periods, while other reports suggest that the benefits are more apparent at an earlier stage (Dusel *et al.*, 1998; Fontes *et al.*, 2004; Gao *et al.*, 2008). During the early ages in broilers, the production

of endogenous digestive enzymes is generally low and may limit feed digestion (Nitsan *et al.*, 1991; Dunnington and Siegel, 1995).

## **2.6 The use of humic acid as a dietary feed additive in broiler chickens**

### *2.6.1 Growth performance*

Humic acids (HA) are natural organic compounds formed from the interaction of soil, humus and lignite forming complex mixtures of polyaromatic and heterocyclic chemicals with numerous carboxylic acid side chains (Klocking, 1994; McCarthy (2001). Humic acid is organic and can be used as poultry feed additives. The inclusion of humates in feed and water of poultry has been reported to promote growth (Shermer *et al* 1998; Karaoglu *et al* 2014). Carcass yield and general characteristics have also been shown to be improved (Kocabagli *et al.*, 2002). Humic acid in broiler diets has the ability to stabilize the intestinal flora and ensures an improvement in utilization of nutrients in poultry diets, hence they have been touted as alternative replacements for antibiotics (Chaveerach *et al.*, 2004) due to their influence on increasing the absorption of mineral components which are essential for the animals. However, Ceylan *et al.*, (2003) and Rath *et al.*, (2006) reported that HA supplementation in chickens' diet may reduce feed intake of broilers if added in large quantities. Generally, humic acid inclusion in diets was observed to improve nutrients utilization, growth and feed conversion efficiency (Lückstädt and Mellor, 2011; Humin Tech, 2004) and also protects the young chicks against competitive exclusion (Mansoub *et al.* 2011). In other studies, it has been reported that humates added to the feed of poultry promote the meat quality measures particularly pH, temperature and colour parameters of breast and thigh muscles for the benefit of consumers (Parks *et al.*, 1996; Karaoglu *et al.*, 2004; Yoruk *et al.*, 2004; Ozturk *et al.*, 2010).

### 2.6.2 Blood parameters and immune system

A few studies have attempted to assess the influence of humic acid on hematological characteristics (white blood cell (monocyte and lymphocyte counts), red blood cell or hematocrit values) (Rath *et al.*, 2006). The results obtained in these studies are inconclusive. Moreover, the studies were conducted in a variety of animal species other than broilers. Nevertheless, as with other organic acids, humic acids may significantly influence hematopoiesis. Some *in vitro* studies showed the ability of HA to activate blood neutrophils and increase their adhesibility (Riede *et al.*, 1991; Chen *et al.*, 2002). In other studies, compounds of similar nature were observed to reduce the hematological disorders associated with aflatoxins and mycotoxins in feeds (Abdel-Wahhab and Aly, 2005; Ozturk *et al.*, 2012). Generally, hematological indices are indicative of the health status of an animal. The white blood cell ratios could be used as reliable biomarkers that indicate any inflammations due to feed induced stress.

Serum biochemical indices normally reflect the condition of an animal and changes due to internal and exogenous factors (Toghyani *et al.*, 2010). Measuring these parameters is significant with regard to monitoring the general health and nutritional status of broilers. In general, higher levels of liver enzymes above the normal ranges indicates liver damage (hepatocellular degeneration) (Badari *et al.*, 2003) brought about probably by free radicals from toxic feed substance. This can be manifested in reduced blood flow (ischemia) to the liver (Khajali and Slominski, 2012). Similar to other organic acids humic acids have been observed to have antioxidative, immunostimulatory and hepatoprotective abilities that allows them to decrease the effects of free radicals brought by increased amounts of toxins with damaging effects on the liver (Abdel-Wahhab and Aly, 2005; Hern'andez *et al.*, 2006; Ozturk *et al.*, 2012).

The immune system of poultry is complex and is composed of several cells and soluble factors that must work in synergy to activate a protective immune response (Khan and Iqbal, 2016). The bursa of Fabricius and the thymus are central lymphoid tissues, peculiar to chickens, which are involved in immune responses. Several studies have demonstrated the importance of organic acids such as humic acid in immune response. Abdel-Fattah *et al.* (2008), Ghazala *et al.* (2011) and Houshmand *et al.* (2012) observed that inclusion of organic acids in poultry diets resulted in heavier immune organs (bursa of Fabricius and the thymus) and also elevated levels of globulin in their serum, an indicator for measuring the immune response. Nevertheless, more studies have to be carried out to validate the influence of humic acid on the general health of broilers.

### *2.6.3 Histomorphology of internal organs and intestines*

Good gut and intestinal health in broiler production is critical for optimisation of feed utilisation efficiency and ultimately for growth rates. Generally, humic acid inclusion in diets have been observed to induce intestinal morphological modifications, increasing the mucosal and cellular permeability (Stepchenko *et al.*, 1991). Previous findings from other studies with compounds of similar nature demonstrated an increase in villus height and crypt depth with inclusion of organic acids in broiler diets (Kum *et al.*, 2010; Rodríguez-Lecompte *et al.*, 2012). The trophic effect of the organic acids such as humic acid could be significant in the stimulation of proliferation of normal crypt cells, promoting healthy tissue development and maintenance (Leeson *et al.*, 2005; Panda *et al.*, 2009). Moreover, the presence of humic acid may contribute in reducing the ability of bacteria to colonize the intestinal mucosa and facilitate increased efficiency of metabolic processes as observed in other studies (Khan, 2013). However, this needs further investigation.

#### 2.6.4 Carcass characteristics, meat quality and fatty acid profiles

Various parameters are used in the measurement of nutritional value of meat including carcass characteristics, instrument-based quality measurements and assessment of fatty acid profiles. All these measures are affected by factors such as diet, age, breed and sex. Dietary influences on nutritional value of meat have been extensively explored (Kissel *et al.*, 2009; Sabow *et al.*, 2015). Nevertheless, gaps still exist on the influence of natural feed additives such as humic acid on nutritional value of broiler meat. The available information on the effects of humic acid on meat quality is largely inconsistent. Le *et al.*, (2016) reported that humic acid inclusion in broiler diets can improve digestion dynamics and nutrient absorption ultimately regulating growth rates and altering the metabolic processes that enhance meat quality traits. Kocabagli *et al.* (2002) and Ozturk *et al.* (2012) also demonstrated a linear increase in body and carcass weights with inclusion of humic acids in the broiler diet. Although the underlying mode of action is still not well understood, humic acid salts have been associated with some meat quality parameters (Berg *et al.*, 2001; Wang *et al.*, 2008; Ozuturk *et al.*, 2011). In chicken and pork, humic acid salt was observed to desirably modify meat colour mainly due to accelerated myoglobin synthesis (Ozuturk *et al.*, 2011).

With regards to fatty acids, several organic acids have been observed to desirably influence the fatty acids profiles of meat (Wang *et al.*, 2008). In pork, humic acid was observed to have an effect of increasing the fat marbling values and to reduce back fat thickness probably due its influence on protein and lipid distribution (Wang *et al.*, 2008). However, the influence of humic acid on fatty acids profiles in broilers is still unknown. Although desirable in meat, a high degree of poly-unsaturation may accelerate a cascade of oxidative processes that promotes deterioration in meat flavour, colour, texture and nutritional value (Mielnick *et al.*, 2006).

Engberg *et al.*, (1996) noted that when the PUFAs are at higher level in muscle membranes, in the presence of light or oxygen they stimulate an upturn in the susceptibility to a cascade of oxidative reactions of the lipids which damages the chemical composition and organoleptic physiognomies and ultimately shortens the shelf-life of the meat and meat products. Consequently, the resultant free radicals (peroxyl and hydroxyl radicals) produced by the process of lipid peroxidation may orchestrate mutagenesis, carcinogenesis and aging in human body systems (Fasesease *et al.*, 2007; Qwele *et al.*, 2013) To scavenge for the free radicals produced, desirable natural antioxidants such as hamates may be critical. Hamates occur naturally as hydrocarbons and contain aromatic and heterocyclic structures, carboxyl groups, and nitrogen, with many active hydrogen bonding sites making them very chemically reactive, raising their potential as antioxidant agents.

#### *2.6.5 Bone development and associated bone diseases*

In broiler chickens (meat-type) selection for rapid growth over a short production cycle has inadvertently resulted in high incidences of immune deficiency and bone disorders such as tibia dyschondroplasia, rickets and associated valgus-varus deformities leading to lameness (Flemming, 2008; Dinev, 2012b). Bone conditions related to weakness of legs have been identified as a severe problem in broiler chickens that really grow fast, causing low economic profits due to a decline in productive efficiency which is brought about by mortality and culling as well as raising concerns about the welfare of the chickens (Ruiz-Feria *et al.*, 2014).

Bone complications are some of the main health issues on broiler chickens and poultry breeders. Broiler skeletal weakness is associated with bone deformities, leg breakage and osteoporosis causing poor performance on broiler chickens. The causes of weakness in the legs and reduced locomotion in broilers are not well understood, since it is a very complex situation

involving several issues such as genetic changes, management, amino acid metabolism, fast growth rate and nutritional insufficiencies, by way of pathological conditions of various organs (Kestin *et al.*, 2001; Fleming, 2008; Tatara, 2009; Tykałowski *et al.*, 2010). The tibia structure is independent on the development of the broiler chicken. Bone formation is vital during early growth and is highly reliant on nutrition.

The current poultry production practises often emphasise provision of diets balanced for Ca, P and vitamins among other important nutrients. Nevertheless, inclusion of non-conventional feed additives such as humates with minute quantities of extra minerals may ensure effective release and assimilation of nutrients and minerals in the gut, stimulating efficient nutrient utilization and more importantly, active bone growth (Scholtz *et al.*, 2007; Emami *et al.*, 2013). Moreover, humic acids can stimulate alterations in intracellular divalent calcium levels and act as dilators increasing the cellular permeability. The increased permeability enhances easier flow of minerals from the blood to the bone and cells (Stepchenko *et al.*, 1991). Nevertheless, information on the influence of humates on bone development in broilers is speculative and hence requires comprehensive investigation.

## **2.7 Summary**

Canola meal is known to be a high quality product and it can be used to reduce feed costs for animal producers. Humic acids and enzyme supplementation are novel natural, organic compound combinations which need to be incorporated in animal feed. The inclusion of these feed additives could promote superior feed utilisation efficiency that may induce significant improvement on growth and meat quality of chickens and hence can be used as alternatives to the antibiotics. The use of Canola, enzymes and humates in diets could also provide an effective escape route from the rising cost of soybean meal, consequenting greater flexibility in the

formulation of low cost diets for broilers. This review has therefore provided basis of the need to explore the use of potassium humate and enzyme as options to improve the utilization of canola-based poultry diets.

## 2.8 References

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## CHAPTER 3

### EFFECT OF HUMIC ACID AND AN ENZYMES ON GROWTH PERFORMANCE, PROTEIN UTILIZATION EFFICIENCY AND BLOOD PARAMETERS OF BROILER CHICKENS FED CANOLA MEAL-BASED DIETS

#### Abstract

The objective of the current study was to investigate the effect of humic acid and enzyme complex as feed additives on growth performance, protein utilization efficiency and blood parameters in broilers fed canola-based diets. Two hundred and twenty broiler chickens were randomly allotted to 5 dietary treatments: Control (commercial broiler diet); CM (17.5 % canola meal inclusion); CMenz (17.5% CM inclusion + 0.3 g/kg Axtra XAP); CMPh (17.5% CM inclusion + 1.5% Potassium Humate, PH) and CMenzPh (17.5% CM inclusion + 1.5% PH + 0.3 g/kg Axtra XAP). Each treatment was replicated 4 times with each pen holding 11 birds as the experimental unit. The feeding trial was conducted over 2 feeding phases: grower phase (15 – 28 d) and finisher phase (29 – 42d). There were no significant ( $P > 0.05$ ) differences on ADFI of all treatments for both grower and finisher phases. However, diet significantly ( $P < 0.05$ ) affected ADG for birds in grower phase. In the grower phase, broilers in CM had the higher ADG ( $71 \pm 1.08$  g/d) whilst the control ( $63.75 \pm 1.08$  g/d) had the lowest. On the contrary, the control had the highest FCR of (1.65) whilst CM (1.47) had the lowest. Broilers in CMenzPh consistently had higher values for the cumulative weight gain throughout the feeding period and also had the highest ( $P < 0.05$ ) final weight (2254.4 g). Diet had no effect on all full blood indices except for the total white blood cell and white blood cell differential, which were consistently high in broilers in CMenzPh. With regards to serum metabolites, only aspartate transferase (AST) and sodium were affected. Treatment 2 ( $406.86 \pm 38.07$  IU/L) had the highest levels ( $P < 0.05$ ) of AST followed by CMenz ( $389.86 \pm 38.07$ ) IU/L whilst

CMenzPh ( $254.17 \pm 41.11$  IU/L) had the lowest levels. Additionally, broilers in CMPH ( $150.57 \pm 0.69$  mmol/l) had the highest ( $P < 0.05$ ) serum sodium content. Overall, canola meal, in the presence of enzyme complex and humic acid, was shown to have great potential as an alternative replacement of soybean meal in broiler diets. The findings from the study can, therefore, contribute to the design of low-cost feed formulations that will improve growth performance and health status in poultry farming systems in the future.

### **3.1 Introduction**

Broiler rations are often formulated from maize grain, known to be an excellent energy source and soybean meal, which has a balanced amino acid profile thus contributing high quality protein to the bird (Opalinski *et al.*, 2006). However, soybean is becoming expensive due to the need to meet both the human and animal demands for the protein source. Consequently, there is a need to explore other protein sources that can practically be included in broiler rations, allowing for least cost ration formulation. Among other protein sources, canola meal has been suggested as an alternative protein source (Wickramasuriya *et al.*, 2015) despite its nutrient composition being relatively lower than that of soybean meal. In addition, the high fibre content in canola meal appears to offset the nutritional benefits that may be realized from using canola meal in chicken diets.

Digestibility of high fibre and secondary plant metabolites (glucosinolates and sinapine) ingredients such as canola can be improved by the use of exogenous enzyme complexes such as Aextra XAP, an enhanced combination of xylanase, amylase and protease, providing vital flexibility in poultry diet formulations. Ultimately, the use of exogenous enzymes in canola diets can increase digestibility, general health status and performance of the animal and thus making the use of canola more economical (Angel *et al.*, 2011). The use of exogenous enzymes

as feed additives to improve feed utilisation can be complemented by inclusion of natural organic acids such as humates in place of conventional antibiotic growth promoters. The enzyme-humic acid combination may offer a potential alternative to the use of conventional antibiotic growth promotants, which have been implicated in the rise of antibiotic resistant super bugs and residues in meat products. Worldwide, there has been a general belief that supplementation with humic substance (HS), as a growth stimulating agent, can have multiple health and nutritional benefits for domestic animals (Ozturk *et al.*, 2012). Moreover, natural additives such as humic acid do not result in the accumulation of harmful residues in meat products (Yoruk *et al.*, 2004) nor do they promote widespread antimicrobial resistance.

The use of organic acids such as humic acid as dietary supplements has the ability to increase the feed conversion ratio (FCR) and average daily gain (ADG) in broiler chickens (Eren *et al.*, 2000; El-Husseiny *et al.* 2008; Kocabağlı *et al.*, 2002). Additionally, organic acids have growth stimulating properties hence they are used as alternatives to antibiotics (Fascina *et al.*, 2012). Moreover, reduced mortality rate (Eren *et al.*, 2000) and improved general health status of broilers (measured by blood metabolomics) can be some of the effects of humic acid (Karaoglu *et al.*, 2004; Yoruk *et al.*, 2004; Ji *et al.*, 2006). In spite of the great potential of canola meal, exogenous enzyme complexes and humic acid as ingredients in formulations of low-cost diets, there is generally a lack of information on their simultaneous use in poultry production. Therefore, the objective of this study was to determine the influence of enzymes (Axta XAP) and potassium humate supplementation on growth performance and blood parameters of broilers fed canola-based diets.

## **3.2 Material and Methods**

### *3.2.1 Study site*

The study was conducted at the North-West University experimental farm (Molelwane). The study site is located in North-West province of South Africa. The geographical coordinates are 25° 28' 0" South, 22° 28' 0" East. The study area is ~ 920 – 1782 metres above sea level. Temperatures range from 3°C - 37°C and rainfall ranges between 300 and 500 mm annually.

### *3.2.2 Feed components*

The potassium humate was obtained from Nutrico (Kempton park, SA) whilst a commercial enzyme complex (xylanase, amylase and protease) Aextra XAP was obtained from Opti feed, SA. Canola meal was obtained from Southern Oil (PTY) LTD, Western Cape and Soybean meal from Opti Feeds, Lichtenburg (SA).

### *3.2.3 Experimental design*

A total of two hundred twenty day old chicks (Cobb 500) obtained from Mimosa Chicks (Mafikeng, SA) were randomly allotted to 5 dietary treatments replicated 4 times with a pen housing 11 birds as the experimental unit. The study was arranged in a completely randomized design. The pens (measuring 3.5 x 1.0 x 1.85 m) were designed to meet the animal welfare standards for optimum production of broilers.

### *3.2.4 Dietary treatments*

The control was a commercial diet whose major protein source was 100% soybean (SBM), whilst the other four diets contained 17.5% canola meal (CM) in place of SBM. Five dietary treatments were formulated as follows: 1. Control (commercial broiler diet); 2. CM (17.5 % canola meal inclusion); 3. CMenz (17.5% CM inclusion + 0.3 g/kg Aextra XAP); 4. CMPH

(17.5% CM inclusion + 1.5% Potassium Humate, PH) and 5. CMenzPh (17.5% CM inclusion + 1.5% PH + 0.3g/kg Aextra XAP). Ingredients and dietary formulations are shown in Table 3.1 whilst Table 3.2 shows the nutritional compositions of the diets.

### *3.2.5 Animals management*

On the day of arrival, the chicks were placed in pens measuring 3.5 x 1.0 x 1.85 m in a broiler house. During the first 3 days of brooding the ambient temperature in the house was kept between 32.5 and 33°C but was gradually reduced reaching 26°C at 14 days of age. These temperature requirements were met using infra-red lights that were used until day 14. Stress packs were given to the chicks for 3 days. The birds were phase-fed starting with the provision of starter ration from day 1 to 14. Experimental diets were only offered during the grower (d 15-28) and finisher (d 29-42) phases. Water was provided ad-libitum. Experimental diets were formulated according to the commercial feed formulation standards to meet the nutrient requirements for the grower and finisher phases. The experimental procedures were approved by the MAREC Animal Research Ethics Committee of North-West University and the Ethics number granted is NWU-00516-16-S9.

### *3.2.6 Feed intake and growth performance*

Feed intake was measured daily and weight gain was measured weekly. All birds from the twenty pens were weighed at the beginning of the trial at d 14 (initial body weight) and subsequently weighed weekly (21, 28, 35 and 42 day) using (TSW equipment weighing scales/Adam equipment). The feed offered was weighed before feeding and refusals were collected each morning before feeding and weighed.

Table 3. 1. Ingredients composition of experimental diets for grower and finisher broilers.

Ingredients	Dietary treatments									
	Grower					Finisher				
	Control	CM	CMenz	CMPh	CMenzPh	Control	CM	CMenz	CMPh	CMenzPh
Yellow Maize-Fine	69.9	59.5	59.5	59.5	59.5	76.2	66.4	66.4	66.4	66.4
Canola oilcake (HEX)	0	17.5	17.5	17.5	17.5	0	17.5	17.5	17.5	17.5
Prime Gluten 60 (Yellow)	1.8	2.4	2.4	2.4	2.4	1.27	1.8	1.8	1.8	1.8
Fullfat Soya	5.1	17.4	17.4	17.4	17.4	1.53	11.7	11.7	11.7	11.7
Soybean Meal (Local)	19.7	0	1.22	1.22	1.22	18	0	0	0	0
Limestone Powder-Fine	1.45	1.22	1.22	1.22	1.22	1.30	1.07	1.07	1.07	1.07
MCP/Mono Cal KK	0.72	0.56	0.56	0.56	0.56	0.50	0.33	0.33	0.33	0.33
Salt-Fine	0.32	0.32	0.32	0.32	0.32	0.33	0.33	0.33	0.33	0.33
Koeksoda	0.17	0.16	0.16	0.16	0.16	0.13	0.12	0.12	0.12	0.12
Choline Powder	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
Lysine	0.28	0.29	0.29	0.29	0.29	0.26	0.27	0.27	0.27	0.27
L-Threonine	0.04	0	0	0	0	0.03	0	0	0	0
Methionine	0.19	0.18	0.18	0.18	0.18	0.16	0.09	0.09	0.09	0.09
PX P2 Br Gr with Phytase	0.17	0.17	0.17	0.17	0.17	0	0	0	0	0
PX P3 Br Fin with Phytase	0	0	0	0	0	0.17	0.17	0.17	0.17	0.17
Coxistac	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Olaquinox	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
Axtra XAP (g/kg)	0	0	0.3	0	0.3	0	0	0.3	0	0.3
Potassium humate (%)	0	0	0	1.5	1.5	0	0	0	1.5	1.5

Gr= Grower, Fin = Finisher, Br = Broiler. Control (commercial broiler diet); CM (17.5 % canola meal inclusion); CMenz (17.5% CM inclusion + 0.3 g/kg Axtra XAP); CMPh (17.5% CM inclusion + 1.5% Potassium Humate, PH) and CMenzPh (17.5% CM inclusion + 1.5% PH + 0.3 g/kg Axtra XAP).

Table 3. 2. Nutrient composition (kg) of experimental diets for grower and finisher broilers.

Parameters	Standard broiler diet composition		Canola oil cake diet composition	
	Grower	Finisher	Grower	Finisher
Volume	100	100	100	100
Moisture	10.93543	11.27404	11.345062	11.579352
ME	11.79982	11.91972	12.099266	12.29989
Protein	18.93943	16.89748	18.000643	16.005623
Fat	6.244323	5.389549	4.161664	3.668489
Fat Ah	7.142858	6.287175	4.887339	4.403945
Fibre	4.175833	4.027193	2.314995	2.205758
Ash	4.845047	4.254113	4.815068	4.218272
Linoleic	2.970173	2.527973	2.003169	1.750331
Choline	1285.453	1159.593	1483.75833	1373.79636
Ca	0.850021	0.750026	0.850056	0.750005
P	0.562513	0.501338	0.496865	0.435018
Na	0.180022	0.169984	0.179983	0.170003
Cl	0.3	0.299997	0.299991	0.3
K	0.732957	0.655133	0.76275	0.684109
ARG	1.10153	0.959095	1.100416	0.957639

ME, metabolizable energy; Ca, calcium; P, phosphorus; Na, sodium; Cl, chlorine; K, Potassium; Arg, arginine.

The average daily feed intake (ADFI), average daily gain (ADG) and feed conversion ratio (FCR) for each feeding phase were calculated as:

$$\text{ADFI} = \frac{\text{Feed offered} - \text{feed refused}}{14 \text{ days}} \quad (1)$$

$$\text{ADG} = \frac{\text{Finish weight} - \text{Start weight}}{\text{Age (days)}} \quad (2)$$

$$\text{FCR} = \frac{\text{feed intake (g)}}{\text{weight gain (g)}} \quad (3)$$

### 3.2.7 Protein utilization efficiency

Protein consumed (PC g/bird) was calculated by multiplying the concentration of crude protein (CP<sub>d</sub>) in the diet (g/kg DM consumed) by feed intake over the feeding phase, whilst protein efficiency ratio (PER g/kg) was calculated by dividing mean body weight gain (BWG) by the mean protein consumed. Specific growth rate (SGR), which is percent growth per feeding phase and growth efficiency (GE) were also calculated using the following formulas:

$$\text{PC} = \text{FI} \times \text{CP}_d \quad (4)$$

$$\text{PER} = \frac{\text{BWG}}{\text{PC}} \quad (5)$$

$$\text{SGR} = \frac{(\text{In final weight} - \text{In initial weight})}{14 \text{ d}} \times 100 \quad (6)$$

$$\text{GE} = \frac{\text{BWG}}{\text{Initial weight}} \quad (7)$$

### 3.2.8 Blood collection and analysis

From each pen, 2 broilers were chosen randomly for blood collection at 40 days of age. Blood was collected from the brachial vein using needle and syringe, and then transferred into two types of tubes. The blood samples were taken to Lancet laboratory (Mafikeng, SA) within 2 h of collection for blood analysis. An anti-coagulant was used for haematological analyses using purple tube so that the blood does not clot. The Idexx lasercyte (Haematology analyser) was used to analyse for haematocrit, haemoglobin, erythrocyte, leucocyte, neutrophils, lymphocytes, monocytes, eosinophil and normoblasts. For serum biochemical indices, the tube without anticoagulant for serum (red tube) was used. The enzymes were analyzed using a clinical chemistry analyser (Gilford Impact, 404IE, Ciba Coming Diagnostic Corp., Gilford Systems, Oberlin, OH 44774). A UV–VIS spectrophotometer (SPECORD 50 PC, Analytik Jena AG) was used to perform the enzyme assays using respective commercial kits (Ciba Coming Diagnostic Corp., Gilford Systems, Oberlin, OH 44774) according to the procedures outlined previously by Ogunsanmi *et al.* (1994). The total protein (TP) and albumin, cholesterol and mineral content were quantified using an auto-analyser (Hitachi-704, Boehringer Mannheim Ltd, Germany).

### 3.2.9 Statistical analysis

Data on growth, protein utilisation efficiency, haematology and serum biochemistry parameters measured were analysed using GLM procedure of SAS (2010) with diet as the only fixed effect (model 1). Data on cumulative weight gain was measured on a weekly basis and were analysed using mixed model procedure of SAS (2010) that took into consideration the effect of both diet and week of measurement (model 2). The PDIFF option of SAS (2010) was used to perform pairwise comparisons of the least square means.

The statistical models were as follows:

$$Y_{ij} = \mu + T_i + \varepsilon_{ij} \quad (1)$$

Where:  $Y_{ij}$  = observation (growth parameters and blood parameter),  $\mu$  = population mean constant common to all observations,  $T_i$  = effect of diet, and  $\varepsilon_{ij}$  = random error term.

$$Y_{ijk} = \mu + T_i + W_j + (T * W)_{ij} + \varepsilon_{ijk} \quad (2)$$

Where:  $Y_{ij}$  = observation (Cumulative weight gain parameter),  $\mu$  = population mean constant common to the observation,  $T_i$  = effect of diet,  $T*W_{ij}$  = effect of diet interacting with week and  $\varepsilon_{ij}$  = random error term. For all tests, the level of significance was set at ( $P < 0.05$ ).

### 3.3 Results

#### 3.3.1 Feed intake and growth performance

The effects of potassium humate and Axtra XAP inclusion in canola diets on growth parameters of broilers at different feeding phases are presented in Table 3.3. The dietary treatment had no influence on ADFI in both grower and finisher phases. Similarly, dietary treatment had no effect on ADG and FCR of the broilers in the finisher feeding phase. On the contrary, significant ( $P < 0.05$ ) differences in ADG and FCR of broilers in the grower phase were observed. In the grower phase, broiler chickens fed CM (Basal diet + 17.5% CM) had the highest ( $71 \pm 1.08$  g/d) ADG ( $P < 0.05$ ) whilst those in the control (normal diet) had the lowest ( $63.75 \pm 1.81$ g/d). In contrast, broilers in the control ( $1.65 \pm 0.04$ ) had the highest ( $P < 0.05$ ) FCR whilst those in CM had the lowest ( $1.47 \pm 0.04$ ). Nevertheless, apart from the control, no difference in ADG and FCR were observed between all other treatments in the grower phase

The cumulative weight gain (CWG) of broilers over the experimental period is presented in Figure 3.1. A clear linear increase in weight gain was observed in both the grower and finisher feeding phases for all the diets except for CM.

Table 3. 3. The effect of enzyme complex and humic acid inclusions on growth performance of broilers fed canola based diets

Parameters	Dietary treatments					SEM
	Control	CM	CMenz	CMPh	CMenzPh	
<i>Grower phase</i>						
ADFI (g)	105.28	104.07	105.64	105.13	106.25	1.81
ADG (g/d)	63.75 <sup>a</sup>	71.00 <sup>b</sup>	66.86 <sup>a<sup>b</sup></sup>	69.35 <sup>b</sup>	68.35 <sup>b</sup>	1.08
FCR	1.65 <sup>b</sup>	1.47 <sup>a</sup>	1.58 <sup>ab</sup>	1.52 <sup>ab</sup>	1.56 <sup>ab</sup>	0.04
<i>Finisher phase</i>						
ADFI (g)	182.91	178.17	185.76	181.91	182.57	3.26
ADG (g/d)	93.69	81.16	90.97	88.84	92.68	4.77
FCR	1.95	2.31	2.05	2.05	1.98	0.15

<sup>a, b</sup> means in the same row with different superscripts are significantly different ( $P < 0.05$ ).

ADFI = Average daily feed intake, ADG = Average daily gain, FCR = Feed conversion ratio. Control (commercial broiler diet); CM (17.5 % canola meal inclusion); CMenz (17.5% CM inclusion + 0.3 g/kg Axtra XAP); CMPh (17.5% CM inclusion + 1.5% Potassium Humate, PH) and CMenzPh (17.5% CM inclusion + 1.5% PH + 0.3 g/kg Axtra XAP).

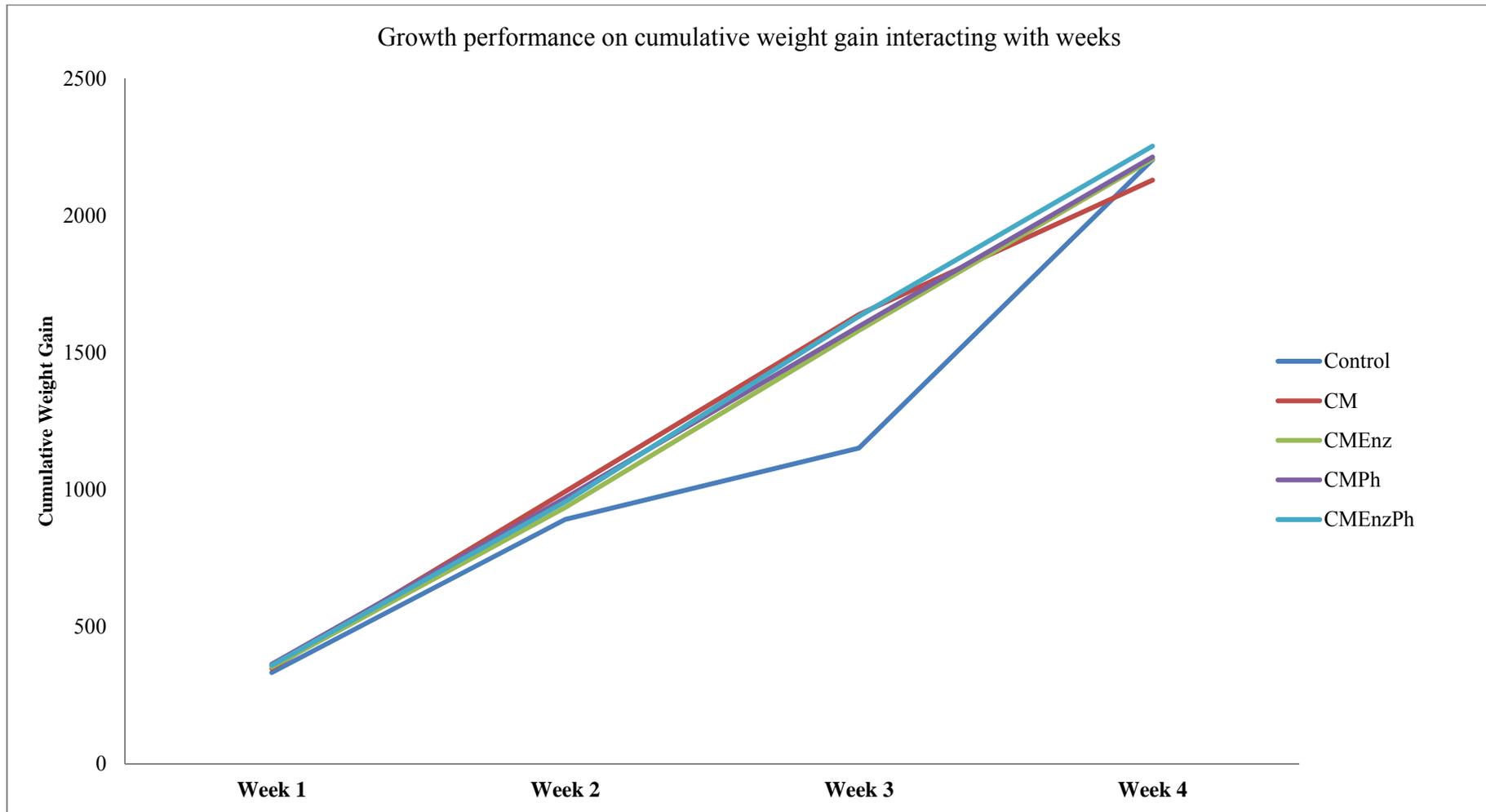


Figure 3. 1 Effect of potassium humate and Aextra XAP on cumulative weight gain on broilers chickens fed canola based diet.

The CWG of the control appeared to be depressed between week 2 and week 3 although it increased sharply thereafter. Among all treatments, the control (333.83 g/w) had the lowest CWG compared to all the other treatments at the beginning. No differences in CWG were observed for broilers fed CM and CMenzPh. Nevertheless, broilers in CMenzPh had the highest values of CWG throughout the entire feeding period. Broilers fed CM (2130.9 g) had the lowest final weight whilst those fed CMenzPh (2254.4 g) had the highest.

### *3.3.2 Protein utilization and growth efficiency*

The results of protein utilization and growth efficiency are presented in Table 3.4. Dietary treatment significantly ( $P < 0.05$ ) affected protein utilisation and growth efficiency parameters in both grower and finisher phases apart from the protein consumed (PC) in the finisher phase. In the grower phase, the PC was highest in CMenzPh (20.12 g) followed by CMenz. However, CM and CMPH had the highest ( $P < 0.05$ ) protein efficiency ratio (PER) whilst the control had the lowest. Specific growth rate was also higher in CM than all other treatments. In all instances, the control had the lowest values for PC, PER, specific growth rate (SGR) and growth efficiency (GE) in the grower phase. On the contrary, in the finisher phase, although the control had the lowest value for PC, the values for PER, SGR and GE were higher ( $P < 0.05$ ) than all other treatments.

### *3.3.3 Haematology parameters*

The full blood parameters are presented in Table 3.5. As illustrated on the table, treatment had no effect on packed cell volume (PCV), haemoglobin (Hb) and the total red blood cell (RBC) count. However, dietary treatment significantly ( $P < 0.05$ ) affected the total white blood cell count (WBC) and all WBC differential counts.

Table 3. 4 The effect of enzyme complex and humic acid inclusions on protein utilization efficiency of broilers fed canola based diets

Parameters	Dietary treatments					SEM
	Control	CM	CMenz	CMPh	CMenzPh	
<i>Grower phase</i>						
PC (g)	18.95 <sup>a</sup>	19.79 <sup>ab</sup>	20.00 <sup>b</sup>	19.91 <sup>ab</sup>	20.12 <sup>b</sup>	0.32
PER	3.36 <sup>b</sup>	3.60 <sup>b</sup>	3.34 <sup>a</sup>	3.88 <sup>b</sup>	3.41 <sup>ab</sup>	0.09
SGR (% day <sup>-1</sup> )	8.63 <sup>a</sup>	9.22 <sup>b</sup>	9.06 <sup>b</sup>	8.95 <sup>a</sup>	9.04 <sup>b</sup>	0.16
GE	0.70 <sup>a</sup>	0.72 <sup>b</sup>	0.72 <sup>b</sup>	0.71 <sup>ab</sup>	0.72 <sup>b</sup>	0.01
<i>Finisher phase</i>						
PC (g)	14.99	13.71	15.33	15.00	15.65	0.80
PER	6.25 <sup>b</sup>	5.92 <sup>a</sup>	5.92 <sup>a</sup>	5.92 <sup>a</sup>	5.92 <sup>a</sup>	0.00
SGR (% day <sup>-1</sup> )	5.05 <sup>b</sup>	4.27 <sup>a</sup>	4.86 <sup>ab</sup>	4.64	4.84 <sup>ab</sup>	0.19
GE	1.03 <sup>b</sup>	0.84 <sup>a</sup>	0.98 <sup>ab</sup>	0.91 <sup>ab</sup>	0.98 <sup>ab</sup>	0.04

<sup>a, b</sup> Means in the same row with different superscripts are significantly different ( $P < 0.05$ ). PC = Protein consumed, PER = Protein efficiency ratio, SGR = Specific growth rate, GE = Growth efficiency. Control (commercial broiler diet); CM (17.5 % canola meal inclusion); CMenz (17.5% CM inclusion + 0.3g/kg Axtra XAP); CMPh (17.5% CM inclusion + 1.5% Potassium Humate, PH) and CMenzPh (17.5% CM inclusion + 1.5% PH + 0.3g/kg Axtra XAP).

Table 3. 5 The effect of enzyme complex and humic acid inclusion on hematology of broilers fed canola-based diets

Parameters	Dietary treatments				
	Control	CM	CMenz	CMPh	CMenzPh
PCV (l/l)	0.36±0.02	0.29±0.02	0.34±0.02	0.33±0.02	0.32±0.02
Haemoglobin (g/dl)	9.71± 0.44	7.91±0.50	9.29±0.50	8.70±0.05	8.58±0.54
Red blood cell (x 10 <sup>12</sup> /l)	2.85 ± 0.14	2.37 ± 0.15	2.67± 0.15	2.68 ± 0.15	2.66± 0.17
White blood cell (x 10 <sup>9</sup> /l)	31.3±4.80 <sup>b</sup>	23.71±5.45 <sup>a</sup>	22.06±5.45 <sup>a</sup>	32.8±5.45 <sup>b</sup>	34.91±5.88 <sup>b</sup>
Neutrophils (x 10 <sup>9</sup> /l)	4.86±1.57 <sup>bc</sup>	1.76±1.89 <sup>a</sup>	2.61±1.89 <sup>a</sup>	3.22±1.89 <sup>b</sup>	5.51±2.05 <sup>c</sup>
Lymphocytes (x 10 <sup>9</sup> /l)	22.7±3.73 <sup>b</sup>	18.8±4.23 <sup>a</sup>	16.31±4.23 <sup>a</sup>	23.65±4.23 <sup>bc</sup>	26.08±4.57 <sup>c</sup>
Monocytes (x 10 <sup>9</sup> /l)	0.52±0.42 <sup>a</sup>	0.35±0.47 <sup>a</sup>	1.12±0.47 <sup>c</sup>	0.77±0.47 <sup>b</sup>	1.13±0.51 <sup>c</sup>
Eosinophils (x 10 <sup>9</sup> /l)	2.92±0.93 <sup>b</sup>	2.65±1.05 <sup>b</sup>	1.76±1.05 <sup>a</sup>	2.19±1.05 <sup>b</sup>	1.80±1.14 <sup>a</sup>
Normoblasts (/100WBC)	0.00±0.17 <sup>a</sup>	0.19±0.19 <sup>a</sup>	0.16±0.19 <sup>a</sup>	0.14±0.19 <sup>a</sup>	0.38±0.20 <sup>b</sup>
Neutro: WBC ratio	0.13±0.03	0.08±0.04	0.07±0.04	0.09±0.04	0.13±0.04
Lym: WBC ratio	0.75±0.05	0.79±0.06	0.81±0.05	0.68±0.06	0.74±0.06
Neutro: Lym ratio	0.18±0.08	0.09±0.09	0.09±0.09	0.28±0.09	0.19±0.11

<sup>a, b</sup> Means in the same row with different superscripts are significantly different ( $P < 0.05$ ). PCV=Packed cell volume, WBC= White blood cell, Neutro = Neutrophils and Lym = Lymphocytes. Control (commercial broiler diet); CM (17.5 % canola meal inclusion); CMenz (17.5% CM inclusion + 0.3g/kg Axtra XAP); CMPh (17.5% CM inclusion + 1.5% Potassium Humate, PH) and CMenzPh (17.5% CM inclusion + 1.5% PH + 0.3g/kg Axtra XAP).

Interestingly, broilers fed CMenz had the lowest amount of the WBC differential counts whilst broilers fed CMenzPh consistently had the highest ( $P < 0.05$ ) counts. The neutrophil to WBC, lymphocytes to WBC and neutrophil to lymphocytes ratios were not significantly ( $P > 0.05$ ) affected by the diet.

#### 3.3.4 Serum biochemical parameters

The serum biochemical indices presented in Table 3.6 reflect a lack of significant effect of dietary treatment on liver enzymes and blood minerals apart from AST and sodium. Chickens offered the CM diet ( $406.86 \pm 38.07$  IU/L) had the highest levels ( $P < 0.05$ ) of AST followed by CMenz chickens ( $389.86 \pm 38.07$  IU/L). The CMenzPh chickens had the lowest AST levels ( $254.17 \pm 41.11$  IU/L). The diet also affected the serum concentrations of sodium with CMPh chickens ( $150.57 \pm 0.69$  mmol/l) having the highest ( $P < 0.05$ ) sodium content compared to the control, CM and CMenzPh (147.44, 147.14 and 147.16 mmol/l) chickens, respectively. The diet had no effect on total protein, serum albumin, cholesterol and all other minerals.

### 3.4 Discussions

#### 3.4.1 Growth performance

The lack of dietary treatment effects on the ADFI for both grower and finisher phase suggest that canola inclusion level (17.5%) was within the optimal range and, therefore, did not cause any significant changes in physico-chemical properties of the diets. In addition, this could be due to the fact that the broilers at the grower stage have a fully developed digestive system to cope with the higher fibre and secondary plant metabolites (glucosinolates and sinapine), which characterise the canola meal (Mailer *et al.*, 2008). Canola meal has high fibre and lower protein compared to soybean meal and it has been observed that poorly digestible protein sources can extremely reduce feed intake and result in lower body weight gain of broiler chickens

Table 3. 6 The effect of enzyme complex and humic acid inclusions on serum biochemistry parameters in broilers fed canola based diets

Parameters	Dietary treatments				
	Control	CM	CMenz	CMPh	CMenzPh
ALP (IU/L)	1835.56 ± 217.52	1723.86±246.64	2313.43±246.64	2401.57±246.64	2330.83±266.40
ALT (IU/L)	2.67 ± 0.48	3.00±0.55	3.00±0.55	2.00±0.55	1.50±0.59
AST (IU/L)	288.78 ± 33.57 <sup>ab</sup>	406.86±38.07 <sup>b</sup>	389.86±38.07 <sup>ab</sup>	313.57±38.07 <sup>ab</sup>	254.17±41.11 <sup>a</sup>
Total protein (g/l)	27.89±0.91	26.00±1.02	27.28±1.02	27.14±1.02	26.17±1.10
Sodium (mmol/l)	147.44±0.62 <sup>a</sup>	147.14±0.69 <sup>a</sup>	148.00±0.69 <sup>ab</sup>	150.57±0.69 <sup>b</sup>	147.16±0.75 <sup>a</sup>
Potassium (mmol/l)	5.71±0.29	4.93±0.33	4.58±0.33	5.47±0.33	5.17±0.36
Albumin (g/l)	11.56±0.35	11.14±0.40	11.57±0.40	12.14±0.40	11.33±0.43
Calcium total (mmol/l)	2.52±0.06	2.49±0.07	2.58±0.07	2.54±0.07	2.44±0.81
Cholesterol (mmol/l)	3.05±0.14	2.92±0.16	3.40±0.16	3.35±0.16	2.98±0.18
Magnesium (mmol/l)	0.98±0.03	0.94±0.27	0.93±0.30	1.01±0.30	0.98±0.33

<sup>a, b</sup> Means in the same row with different superscripts are significantly different ( $P < 0.05$ ). ALP =Alanine aminotransferase, ALT =Alkaline phosphatase & AST =Aspartate aminotransferase). Control (commercial broiler diet); CM (17.5 % canola meal inclusion); CMenz (17.5% CM inclusion + 0.3g/kg Axtra XAP); CMPh (17.5% CM inclusion + 1.5% Potassium Humate, PH) and CMenzPh (17.5% CM inclusion + 1.5% PH + 0.3g/kg Axtra XAP).

(Widyaratne and Drew, 2011). However, ADG was improved in canola-containing diets, whilst FCR was lowered. The higher ADG and reduced FCR in canola containing treatments could be due to the additives (humic acid) as well as the phytogetic compounds in CM, which improved the feed efficiency given the reduced feed intake, hence the higher weight gains in the grower phase. Growth promoting agents such as humic acid and phytogetic compounds have been observed to improve digestion dynamics and feed utilization dynamics of poor protein sources in particular (Toghyani *et al.*, 2010; Rath *et al.*, 2006; Ferket and Gernat, 2006).

The results of the current study on broilers fed an enzyme complex, Axtra XAP, for both feeding phases indicated that the feed conversion ratio in broilers fed canola-based diets had improved feed utilization as was observed in other studies (Pucci *et al.*, 2010; Tang *et al.* 2014). Cowieson and Ravindran (2008) also observed that addition of Axtra XAP in broiler diets had a positive influence on the overall performance of broilers. On the contrary, Kocher *et al.* (2003) observed that enzyme complex including (xylanase, amylase and protease) had a slight influence on broiler overall performance. In addition, other researchers observed that addition of an enzyme complex had a negative influence on performance variables (Brufau *et al.*, 2006; Yegani and Korver, 2013). Therefore, it may perhaps be challenging to evaluate the effect of enzymes added to low-protein diets based only on protein digestibility due to lack of change in digestibility for various protein sources. Nevertheless, the positive effects observed in the current study indicate that Axtra XAP was effective in the breakdown of the matrix of cell wall in fibrous canola, thus helping the release of nutrients captured in cell walls due to easier access of digestive enzyme to these substrates as observed in other studies (Eren *et al.*, 2000; Kocabagli *et al.*, 2002; El-Husseiny *et al.*, 2008).

The cumulative weight gain of all treatments was progressively increasing over the feeding period of the study. This agrees with the findings of Bochno *et al.* (2003) who observed that as broilers grow older, the muscle tissue or other tissues increase whilst the bone content declines and the above differences are most noticeable until 6 weeks of age. Gajana *et al.* (2011) reported that broiler chickens raised until week 5 can obtain a final body weight of about 1.7 to 1.9 kg provided that the starter diet is altered, which is different from the results of the current study where the final body weight gain was between 2.13 - 2.24 kg when the starter diet was changed at grower to finisher phase.

#### *3.4.2 Protein utilisation and growth efficiency*

It is clear from the current study that diets with low-protein content generally utilise dietary energy and protein more efficiently than the control diet in line with what was observed by Aletor *et al.* (2000). Findings from the current study indicate that there was an improved protein utilization efficiency in treatments without enzyme complex supplement whilst the diets with inclusion of enzyme complex had a reduced PER. In addition, as the PC level is further reduced PER is increased and this may suggest that more of essential amino acids in the diet become limiting, which result in a reduced GE of the broilers.

#### *3.4.3 Haematology parameters*

Overall, haematological indices were observed to be within the anticipated ranges for healthy broilers (Merck Manual, 2012). This implies that the inclusion of canola meal and the growth promoting agents did not influence haematopoiesis. In fact, the desirable blood metabolites levels realized in the current study indicates the potential of the humic acid and canola meal with phytogetic compounds in improving the general health status of broilers. In other studies, compounds of similar nature were observed to reduce the haematological disorders associated

with aflatoxins and mycotoxins in feeds (Ozturk *et al.*, 2012; Abdel-Wahhab and Aly, 2005). Generally, haematological indices are normally indicative of the health status of animals. In the current study, no effect of diet was observed on the lymphocyte to WBC, neutrophil to WBC and neutrophil to lymphocyte ratios. The white blood cell ratios could be used as reliable biomarkers that indicate any inflammations stimulated by feed induced stress. Currently, there is very limited information on the use of such ratios in assessing the effects of the inclusion of unconventional ingredients in diets of broilers on the general health of animals.

#### *3.4.4 Serum biochemical indices*

The serum metabolites observed were all within normal ranges for healthy broiler chickens (Merck manual, 2012). Serum biochemical indices normally reflect the condition of an animal and any changes in response to internal and exogenous factors (Toghyani *et al.*, 2010). Measuring these parameters is significant in regards to monitoring the general health and nutritional status of broilers. Although AST levels were higher for the birds fed canola diets, the levels were still within the normal ranges. Nevertheless, the observed high levels of AST in CM and CMenz could perhaps be due to an increased liver activity in an attempt to detoxify the higher amounts of secondary plant metabolites in canola. Generally, higher levels of liver enzyme above the normal ranges signify liver damage (hepatocellular degeneration) (Badari *et al.*, 2003). This may also be manifested in reduced blood flow (ischemia) to the liver (Khajali and Slominski, 2012). Interestingly, the AST values in chickens offered potassium humate-containing diets were similar to those of the control chickens. This may be indicative of the hepatoprotective abilities of potassium humate in reducing free radicals induced by increased amounts of secondary plant metabolites in canola that could have caused liver damage (Hernández *et al.*, 2006). As observed in other studies, no variation in protein concentration or total albumin was observed in the current study. Together with other serum metabolites,

protein concentration gives an indication of the adequacy of protein in the diet and the efficiency with which it is being utilised by the broilers (Ghammry *et al.*, 2002). The results therefore imply that the inclusion of canola at 17.5% did not cause any impediments on the protein digestion dynamics and utilisation by broiler chickens. Therefore, canola can be used to replace soybean meal at that level without major effects on blood metabolites.

### **3.5 Conclusion**

Inclusion of humic acid and enzyme complex in diets positively influenced the growth performance, health status and well-being of the broilers fed canola-based diets. Growth performance indices were improved in the canola-based diets due to the effects of growth promoting agents (humic acid and enzymes) that improved feed utilisation efficiency. The low AST levels in chickens fed diets containing humic acid indicates the hepato-protective ability of humic acid in dealing with increased amount of secondary plant metabolites that could have affected the liver function. Overall, canola meal was shown to have great potential as an alternative replacement of soybean meal in broiler diets. Collectively, findings from the study can be helpful in designing low-cost feed formulations that will improve growth performance and health status in poultry farming systems in future. Nevertheless, it may also be interesting to investigate whether the influence of humic acid and enzyme inclusion in diets may be associated with an improvement on meat quality attributes of broiler meat.

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**CHAPTER 4**

**INFLUENCE OF OF HUMIC ACID AND ENZYMES ON CARCASS**

**CHARACTERISTICS, MEAT QUALITY AND FATTY ACID PROFILES IN**

**BROILERS FED CANOLA-BASED DIETS.**

**Abstract**

This study was conducted to assess the effect of potassium humate and Aextra XAP (Xylanase + Amylase + Protease) inclusions in diets on carcass characteristics, meat quality and fatty acid profiles on broilers fed canola-based diets. Two hundred and twenty broilers chickens randomly allotted to 5 dietary treatments: the control (commercial broiler diet); CM (17.5 % canola meal inclusion); CMenz (17.5% CM inclusion + 0.3 g/kg Aextra XAP); CMPh (17.5% CM inclusion + 1.5% Potassium Humate, PH) and CMenzPh (17.5% CM inclusion + 1.5% PH + 0.3 g/kg Aextra XAP) were slaughtered at day 42 for assessment of carcass and meat quality parameters. From the results, diet had no effect on all carcass traits apart from breast muscle weight and breast muscle index. The highest breast muscle weight was observed in broilers fed CMenz ( $487.6 \pm 17.5$  g) followed by those fed the control diet ( $474.37 \pm 17.5$  g). The results on meat quality measurements also showed no significant dietary effect on pH, temperature, drip loss and shear force values of the breast muscle. However, diet had a significant effect on the 3 meat colour coordinates and water-holding capacity (WHC). With regards to meat colour, broiler muscle in the control and CMPh groups (52.94 and 52.91) had the highest ( $P < 0.05$ ) values for lightness ( $L^*$ ), whilst the meat from broilers fed CMenzPh had the lowest value (47.94). In contrast, CM group had the lowest ( $P < 0.05$ ) value for redness ( $a^*$ ) with CMenzPh group having the highest values. The results of WHC showed that breast muscles from the control group (29.34%) with the lowest value had the greatest water holding capability. With regards to fatty acid profile, higher values for PUFAs, n-3 fatty acids and n-6

fatty acids were observed in the CM containing diets particularly the CMPh group. On the contrary, higher values for SFAs were observed in the Control group. The inclusion of CM, enzyme complex and humic acid salt also increased the PUFA/SFA ratio whilst at the same time reducing the n-6/n-3 ratios which are important in assessing the nutritional value of the meat. Findings from the current study can be helpful in designing low-cost feed formulations that can improve carcass characteristics, meat quality and fatty acid profiles for the benefit of consumers.

#### **4.1 Introduction**

Poultry meat is among the cheapest sources of animal protein and is the most preferred and consumed meat followed by pork and beef worldwide (Le Bihan-Duval, 2004). The production and consumption of poultry meat and products increase over the years worldwide due to the associated desirable nutritional properties, particularly the high protein content, low fat and relatively high levels of polyunsaturated fatty acids (PUFAs) when compared to other meat products (Brenes and Roura, 2010). Producing meat and meat products with increased levels of minerals (iron, zinc, and selenium), polyunsaturated fatty acids (PUFA) and antioxidants can be attractive and valued worldwide in the food market in terms of quality, defence and nutritional improvement of foodstuff (Ozbey *et al.*, 2007).

Various factors, including diet, have been observed to affect meat quality particularly meat pH, water-holding capacity (WHC) the ability of the muscle to retain the moisture that is naturally occurring in the meat), marbling, meat tenderness and meat colour, which are important in determining consumer preferences and meat purchasing decisions. In Chapter 3, it was observed that diet influenced growth performance and health of broilers. It can, therefore, be expected that ration composition and inclusion of natural feed additives may have the potential

to alter meat metabolite profiling that may result in desirable meat compositional and organoleptic physiognomic quality (Ozbey *et al.*, 2007).

Research has been done on the effects of enzymes on the performance of broilers that are fed corn and those fed on soybean meal based diets (Cowieson and Ravindran, 2008). In particular, in several studies, digestibility mechanisms and the efficiency of feed utilisation improved when a xylanase, amylase and protease enzyme complex was supplemental to the broiler diet (Persia *et al.*, 2002; Cowieson and Ravindran, 2008; Yuan *et al.* 2008). The available information on the effects of humic acid on meat quality is inconsistent. Despite humic acid salt having been associated with some meat quality parameters (Berg *et al.*, 2001; Wang *et al.*, 2008; Ozuturk *et al.*, 2012), the underlying mode of action is still not well understood. In chicken and pork, humic acid salt was observed to desirably modify meat colour mainly due to accelerated myoglobin synthesis (Ozuturk *et al.*, 2012). Moreover, in pork, humic acid was observed to have an effect of increasing the fat marbling values and to reduce back fat thickness probably due its influence on protein and lipid distribution (Wang *et al.*, 2008).

Information on the interactive effects of enzyme complex and organic acids such as humic acid salt on meat quality traits of broilers fed CM, a poor protein source in place of soybean meal is scarce. Besides providing options for formulation of least cost diets, CM, exogenous enzymes and humic acid salt may desirably alter the meat quality traits for the benefit of consumers. Therefore, the current study was designed to determine the influences of humic acid and enzyme complex on carcass characteristics, meat quality and fatty acid parameters in broilers fed canola-based diets.

## **4.2 Materials and methods**

### *4.2.1 Study site, source of diets, animal management*

The description of the study area is explained in Section 3.2.1-3.2.3 whilst the Section 3.2.4 explains the experimental design and dietary treatments.

### *4.2.2 Slaughter procedures*

At the end of the feeding trial, all broilers in the pens were deprived of feed for a period of 13 hours to allow the clearing of the crop (Ari *et al.*, 2013). Thereafter, all broilers were taken to Rooigrond Braaikuikens abattoir A3/32 (Mafikeng, South Africa) for slaughter. Upon delivery at the abattoir the broilers were grouped according to dietary treatment (Control, CM, CMenz, CMPh and CMenzPh), put into a metal rack that holds them upside down for stunning. Chickens were slaughtered by cutting the jugular vein with a sharp knife and were bled for up to 2 minutes. Subsequently, chickens followed the standard procedures before they were put into a de-feathering machine. After feathers were removed, hot carcasses (without neck, giblets, and feet) were weighed. The carcasses and internal organs were taken back to the NWU farm laboratories for determination of internal organ size and length then the carcasses were kept in a cold room for 24 h after which cold carcass weight, carcass pH and temperature were measured.

### *4.2.3 Carcass traits and internal organs*

After the slaughter, carcasses from the different treatments were identified by placing them in tagged plastics bags for each treatment. Thereafter, carcass evisceration and carcass characteristic measurements were done at the abattoir. The following organs were removed and weighed: gizzards, livers, hearts, spleens, breasts and drumsticks. Length of small intestines (jejunum, duodenum and ileum) were measured and recorded. The carcass weight of each

chicken was recorded and dressing out percentage was calculated. Breast (pectoralis major muscle) samples were collected at 24 hours post mortem for evaluation of meat quality traits. Thereafter, breast samples were carefully removed then vacuum packed and kept frozen ( $-20^{\circ}\text{C}$ ) until meat quality analysis at NWU laboratory and other samples were sent for analysis of fatty acids at the Food Science Division, University of Free State (SA).

#### *4.2.4 Meat pH and temperature*

Meat pH ( $\text{pH}_u$ ) and temperature measurements were taken 24 hours post-slaughter on the breast muscle (central area of the breast) using a Corning Model 4 pH-temperature meter (Corning Glass Works, Medfield, MA) equipped with an Ingold spear-type electrode (Ingold Messtechnik AG, Udorf, Switzerland) (Stanford et al., 2003).

#### *4.2.5 Meat colour*

Colour of the meat ( $L^*$  = Lightness,  $a^*$  = Redness and  $b^*$  = Yellowness) was measured using a Minolta colour-guide (Spectrophotometer CM 2500c, Konika Minolta, Osaka, Japan), with a 20 mm diameter measurement area with innovative  $45^{\circ}$  a: 0 geometry optics. The colour meter was calibrated before measurements using a white tile for standard white colour calibration. Colour recording was done in triplicate of 6 samples of each treatment on the surface of a freshly cut slice of the breast muscle allowed to bloom for 1 hour on a polystyrene tray at  $4^{\circ}\text{C}$ .

#### *4.2.6 Meat water holding capacity*

The WHC of the meat measurements were done in duplicate samples on the surface of a freshly cut slice of the pectoralis major muscle (PMM) (8-16 grams), and was determined as the amount of water expressed from fresh meat held under pressure (60 kg pressure) using the filter-paper press method developed by Grau and Hamm (1957). The water from the fresh meat

was taken up by a pre-weighed filter paper and calculated as a percentage. Water holding capacity was calculated using the equation:

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

#### 4.2.7 Meat drip loss

Drip loss measurement was determined using a method adapted from Zhang *et al.* (2009). Pieces of muscle from the pectoralis major muscle (PPM) weighing ~ 2 grams (wet weight,  $w_1$ ) were hooked and suspended using wire steel in a plastic bottle and sealed properly so that the samples did not touch the sides of the bottle. Bottles were stored in a refrigerator at 4°C for 72 hours. The suspended samples were stored in a cold room at 4°C for 72 hours. The meat samples were reweighed to obtain weight after drip ( $w_2$ ). The difference in weight of each sample before and after drip was conveyed as percentage drip loss and calculated as follows:

$$Drip\ loss (\%) = \frac{(w_1 - w_2)}{w_1} \times 100\%$$

Where;  $w_1$  is initial weight, and  $w_2$  is weight after drip.

#### 4.2.8 Meat cooking loss

Raw breast muscle samples chilled overnight at 4°C chiller were individually weighed to obtain initial weight ( $w_1$ ) of PMM after thawing. The samples were then placed in foil plate and oven broiled (dry heating) at 180°C for 30 minutes. The broiled samples were then removed from the oven and left to cool for 20 minutes. The samples were then re-weighed to obtain the cooked weight ( $w_2$ ) of the PMM). The cooking loss was calculated based on the difference between the weight of raw meat and cooked meat using the following equation:

$$Cooking\ loss (\%) = \frac{(w_1 - w_2)}{w_1} \times 100\%$$

Where:  $w_1$  is weight of raw meat,  $w_2$  is weight after cooking.

#### 4.2.9 Meat tenderness

The breast muscle samples that were previously cooked at 180°C for 30 minutes and used for determination of cooking loss were used for shear force evaluation. The subsamples of 2 cm high × 2 cm width × 12 cm length dimension were sheared perpendicular to the fibre direction using a Meullenet - Owens Razor Shear Blade (A/MORS) mounted on a Texture analyser (TA XT plus, Stable Micro Systems, Surrey, UK). The reported value in Newtons (N) represented the average of the peak force measurements of each sample.

#### 4.2.10 Proximate and fatty acid analysis

Total lipids from muscle samples were quantitatively extracted, according to the method of Folch *et al.*, (1957) using chloroform and methanol in a ratio of 2:1. An antioxidant, butylated hydroxytoluene, was added at a concentration of 0.001 % to the chloroform: methanol mixture. The fat extracts were desiccated under vacuum using a rotary evaporator and subsequently dried in a vacuum oven overnight at 50°C. Phosphorus pentoxide was used as moisture absorbent. Determination of total extractable fat was done gravimetrically from the extracted fat and express as percent fat (w/w)/100g tissue. Pending for fatty acid analyses, the extracted fat from feed, subcutaneous fat and meat were then stored in a polytop (glass vial, with a push-in top) under a blanket of nitrogen and frozen at -20°C.

A lipid aliquot (20 mg) from the, subcutaneous and muscle lipid were transferred into a Teflon-lined screw-top test tube by means of a one-use glass Pasteur pipette. 0.5 M NaOH in methanol and 14% boron trifluoride in methanol was then used to trans-esterify the fatty acids forming fatty acid methyl esters (FAMES) (Park and Goins, 1994). A Varian 430 flame ionization gas chromatography (GC), with a fused silica capillary column, Chrompack CPSIL 88 (100 m length, 0.25 mm ID, 0.2 µm film thicknesses) was used to quantify the Fatty Acid Methyl Esters

(FAMEs) from subcutaneous fat and muscle. The Analysis of FAMEs was done initially using an isothermic period (40°C for 2 minutes) with the temperature being increased thereafter, at a rate of 4°C/minute to 250°C. Finally, an isothermic period at 230°C for 10 minutes followed. The Varian CP 8400 Auto sampler was used to inoculate a volume of 1µl Fatty Acid Methyl Esters n-hexane with the inoculation port and detector maintained at 250°C. The carrier gas used was Hydrogen, at 45 psi, whilst nitrogen was employed as the makeup gas. Recording of the chromatograms was done using the Galaxy Chromatography Software.

Identification of the Fatty acid methyl ester samples was done using the standards attained from Supelco (Supelco 37 Component Fame Mix 47885-U, Sigma-Aldrich Aston Manor, Pretoria, South Africa) through comparisons of the retention times of FAME peaks from samples with those of the standards. All other reagents and solvents were of analytical grade and attained from Merck Chemicals, SA (Pty Ltd, Halfway House, and Johannesburg, South Africa). Individual fatty acids were calculated as a proportion of total fatty acids present in the sample, while total fatty acids combinations were calculated as: omega-3 (n-3) fatty acids, omega-6 (n-6) fatty acids, total saturated fatty acids (SFA), total monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), PUFA/SFA ratio (P/S) and n-6/n-3 ratio.

#### *4.2.11 Statistical analysis*

Data on carcass characteristics, meat quality parameters and fatty acid profiles of broiler meat were analysed using GLM procedure of SAS (2010).

The statistical models were as follows:

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

Where:  $Y_{ij}$  = observation (carcass characteristics, meat quality parameters and fatty acid profiles),  $\mu$  = population mean constant common to all observations,  $T_i$  = effect of diet, and  $\epsilon_{ij}$  = random error term, for all tests, the level of significance was set at ( $P < 0.05$ ).

## **4.3 Results**

### *4.3.1 Carcass traits*

The effects of enzyme complex and humic acid salt dietary inclusion on carcass traits of broiler chickens are presented in Table 4.1. From the results, it can be confirmed or concluded that diet had no effect on all carcass traits apart from breast muscle weight and breast muscle index. The highest breast muscle weight was observed in chickens offered CMenz ( $487.6 \pm 17.5$  g) diet followed by chickens in the control group ( $474.37 \pm 17.5$  g). The CM fed broilers had the lowest breast weight. Similarly, CMenz broilers had the highest breast muscle index followed by the control treatment group. The CMPH fed broilers had the lowest breast muscle index.

### *4.3.2 Internal organs*

The effects of potassium humate and enzyme complex inclusion in canola meal based diets on carcass characteristics measurements of broilers are presented in Table 4.2. The weights of the internal organs (gizzard, heart, spleen) and intestinal length (duodenum, jejunum, ileum) were significantly ( $P < 0.05$ ) influenced by the diets. The gizzard and spleen of broilers in the CMenzPh treatment were heavier ( $P < 0.05$ ) than in broilers offered CMenz. Chickens fed the CMenzPh diet also had the longest small and large intestines compared to the other dietary treatments. The heart weight was heaviest ( $P < 0.05$ ) in CMenz (25.90 g) whilst CMPH and CMenzPh chickens had the lowest heart weights. Diet had no effect on the liver weights and the hepatosomatic index.

Table 4.1 The effect of potassium humate and Aextra XAP inclusions on carcass characteristics and meat quality measurements of broilers fed canola based diets.

Parameters	Dietary treatments					SEM
	Control	CM	CMenz	CMPh	CMenzPh	
Final body weight (g)	2585	2507	2574	2605	2630	74.94
Hot carcass weight (g)	1806.25	1711.25	1852.50	1970	1963.75	106.19
Cold carcass weight (g)	1803	1870	1915.25	1842.75	1883.25	46.09
Dressing %	69.92	69.02	72.05	75.52	74.64	4.42
Av Breast weight (g)	474.37 <sup>b</sup>	422.82 <sup>b</sup>	487.6 <sup>b</sup>	383 <sup>a</sup>	447 <sup>b</sup>	17.5
Av Thigh weight (g)	264.10	269.79	277.61	254.06	277.06	7.48
Breast muscle ratio	0.26 <sup>b</sup>	0.23 <sup>a</sup>	0.27 <sup>b</sup>	0.22 <sup>a</sup>	0.24 <sup>ab</sup>	0.01
Thigh muscle ratio	0.15	0.15	0.15	0.14	0.15	0.01

<sup>a, b</sup> Means in the same rows with different superscripts are significantly different ( $P < 0.05$ ). L\* = lightness, a\* = redness, b\* = yellowness; Control (commercial broiler diet); CM (17.5 % CM inclusion); CMenz (17.5% CM inclusion + 0.3g/kg Aextra XAP); CMPh (17.5% CM inclusion + 1.5% Potassium Humate, PH) and CMenzPh (17.5% CM inclusion + 1.5% PH + 0.3g/kg Aextra XAP).

Table 4.2 The effect of potassium humate and Axtra XAP inclusions on internal organs of broilers fed canola based diets

Parameters	Dietary treatments					SEM
	Control	CM	CMenz	CMPh	CMenzPh	
Gizzard (g)	30.75 <sup>ab</sup>	30.90 <sup>ab</sup>	27.70 <sup>a</sup>	33.10 <sup>bc</sup>	35.15 <sup>c</sup>	1.06
Heart (g)	12.60 <sup>b</sup>	13.55 <sup>b</sup>	12.6 <sup>b</sup>	11.70 <sup>a</sup>	11.52 <sup>a</sup>	4.21
Spleen (g)	2.45 <sup>a</sup>	2.35 <sup>a</sup>	2.85 <sup>a</sup>	4.15 <sup>b</sup>	4.69 <sup>b</sup>	0.18
Duodenum (cm)	38.54 <sup>b</sup>	31.24 <sup>b</sup>	36.36 <sup>b</sup>	25.84 <sup>a</sup>	26.20 <sup>a</sup>	2.69
Jejunum (cm)	41.28 <sup>a</sup>	35.28 <sup>a</sup>	45.10 <sup>a</sup>	64.80 <sup>b</sup>	97.64 <sup>b</sup>	4.87
Ileum (cm)	52.34 <sup>b</sup>	41.70 <sup>a</sup>	53.68 <sup>b</sup>	42.74 <sup>a</sup>	43.34 <sup>a</sup>	3.66
Liver (g)	41.55	42.85	42.69	40.65	40.00	1.27
HSI	0.023	0.024	0.023	0.023	0.022	0.00

<sup>a, b</sup> Means in the same rows with different superscripts are significantly different ( $P < 0.05$ ). HSI = hepatosomatic index. Control (commercial broiler diet); CM (17.5 % CM inclusion); CMenz (17.5% CM inclusion + 0.3g/kg Axtra XAP); CMPh (17.5% CM inclusion + 1.5% Potassium Humate, PH) and CMenzPh (17.5% CM inclusion + 1.5% PH + 0.3g/kg Axtra XAP).

#### 4.3.3 Meat quality

The results on meat quality measurements showed a lack of significant effect of diet on meat ultimate pH, temperature, drip loss and shear force values of the breast muscle (Table 4.3). However, diet had a significant effect on the 3 meat colour coordinates and water holding capacity. With regards to meat colour, broiler muscle in the control and CMPH treatment groups ( $52.94 \pm 0.67$  and  $52.91 \pm 0.67$ ) had the highest ( $P < 0.05$ ) lightness ( $L^*$ ) values, whilst the breast muscle from the CMenzPh fed broilers had the lowest ( $47.94 \pm 0.67$ ). In contrast, CM fed broilers had the lowest ( $P < 0.05$ ) value for redness ( $a^*$ ) whilst the CMenzPh treatment group had the highest value. In terms of yellowness ( $b^*$ ), the highest value ( $P < 0.05$ ) was observed in CMPH (14.30) fed broilers. Results of water-holding capacity (WHC), showed that breast muscles in control (29.34%) group with the lowest value had the greatest WHC followed by the CMenzPh treatment group. However, the highest value for WHC was observed in the CMenz ( $37.47 \pm 1.93$ ) group showing less ability of the breast muscle in retaining water compared to the other treatments.

#### 4.3.4 Proximate fat composition

The effect of potassium humate and Aextra XAP inclusion in CM based diets on proximate fatty acid composition (%) of broiler meat is presented in Table 4.4. Diet had no effect ( $P > 0.05$ ) on the intramuscular fat (IMF). On the contrary, diet had an effect ( $P < 0.05$ ) on the fat free dry matter and moisture content. The broiler chickens fed CMPH diet had higher ( $P < 0.05$ ) fat free dry matter (FFDM) than those that received other dietary treatments. Broilers fed the control and CM diets had similar FFDM whilst CMenz had the lowest FFDM among treatments. The moisture content accumulated in the breast muscle was higher when broilers were fed the CMenz diets and lower when fed the CMPH diet.

Table 4.3 The effect of potassium humate and Aextra XAP inclusions on meat quality measurements of broilers fed canola-based diets.

Parameters	Dietary treatments					SEM
	Control	CM	CMenz	CMPh	CMenzPh	
Ultimate pH	6.12	6.16	6.12	5.95	5.89	0.09
Ultimate Temperature (°C)	14.63	12.56	14.08	15.54	16.69	0.69
<i>Meat colour</i>						
L*	52.94 <sup>b</sup>	50.79 <sup>c</sup>	51.35 <sup>bc</sup>	52.94 <sup>b</sup>	47.94 <sup>a</sup>	0.67
a*	1.81 <sup>a</sup>	1.59 <sup>a</sup>	2.46 <sup>ab</sup>	1.81 <sup>a</sup>	3.42 <sup>b</sup>	0.41
b*	13.91 <sup>a</sup>	13.33 <sup>a</sup>	13.69 <sup>a</sup>	14.30 <sup>b</sup>	13.71 <sup>a</sup>	0.69
Water holding capacity (%)	29.34 <sup>a</sup>	33.62 <sup>ab</sup>	37.47 <sup>b</sup>	34.97 <sup>ab</sup>	33.04 <sup>ab</sup>	1.93
Drip loss (%)	9.58	8.45	9.69	10.91	9.22	1.02
Cooking loss (%)	13.75	14.73	18.07	14.45	12.40	1.47
Shear Force (N)	6.47	6.38	8.32	8.81	8.31	1.07

<sup>a, b</sup> Means in the same rows with different superscripts are significantly different ( $P < 0.05$ ). L\* = lightness, a\* = redness, b\* = yellowness; Control (commercial broiler diet); CM (17.5 % canola meal inclusion); CMenz (17.5% CM inclusion + 0.3g/kg Aextra XAP); CMPh (17.5% CM inclusion + 1.5% Potassium Humate, PH) and CMenzPh (17.5% CM inclusion + 1.5% PH + 0.3g/kg Aextra XAP).

Table 4.4. Effects of potassium humate and Axtra XAP dietary inclusions on proximate fat composition (%) of broiler meat.

Parameter	Dietary treatments					SEM
	Control	CM	CMenz	CMPh	CMenzPh	
IMF	1.67	1.73	1.50	1.31	1.54	0.16
FFDM	22.99 <sup>b</sup>	22.87 <sup>b</sup>	22.11 <sup>a</sup>	24.18 <sup>c</sup>	22.58 <sup>ab</sup>	0.27
Moisture	75.34 <sup>b</sup>	75.40 <sup>b</sup>	76.38 <sup>c</sup>	74.51 <sup>a</sup>	75.87 <sup>b</sup>	0.24

<sup>a, b, c</sup> Means in the same row with different superscripts are significantly different ( $P < 0.05$ ). Parameter: IMF = Intramuscular fat; FFDM = Fat free dry matter. Control (commercial broiler diet); CM (17.5% CM inclusion); CMenz (17.5% CM inclusion + 0.3g/kg Axtra XAP); CMPh (17.5% CM inclusion + 1.5% Potassium Humate, PH) and CMenzPh (17.5% CM inclusion + 1.5% PH + 0.3g/kg Axtra XAP).

#### 4.3.5 Fatty acids profiles

The effects of potassium humate and Aextra XAP inclusions in CM based diets on breast muscle fatty acids profiles are presented in Table 4.5. From the results, the breast muscle from broilers fed the control diet had higher ( $P < 0.05$ ) myristic, myristoleic, palmitic, palmitoleic and oleic in that order. The lowest values were observed in broilers fed CMenz for myristic, CMenz, CMPH and CMPHenz for myristoleic. In all treatments, the highest proportions of fatty acids (FAs) were obtained for palmitic acid, stearic acid, oleic acid and linoleic acid. With regards to linoleic and  $\gamma$ -linolenic acids, broilers fed the control diet had the lowest values, whilst those fed CM diet had the highest values. In addition, the eicosadienoic and eicosatrienoic acids in breast meat of broiler fed CM and CMPH diets were significantly elevated compared to those fed other diets, with the broilers fed the control diet also having the lowest proportions. Similarly, higher proportions of docosapentaenoic and docosahexanoic were obtained in broilers fed CMPH and CM diets, whilst those fed the control diet had the lowest content. Diet had no effect on all other FAs including pentadecylic, margaric, heptadecenoic, nonoadecanoic and eicosatrienoic acids.

#### 4.3.6 Nutritional indices of broiler meat

The effects of dietary inclusion of potassium humate and Aextra XAP in CM-based diets on nutritional indices of broiler meat are presented in Table 4.6. The diet had an effect ( $P < 0.05$ ) on total FAs of the breast muscle. From the results, broilers fed the control and CMPH diets had higher total saturated fatty acids (SFA) whilst those fed CM diet had the lower values. The broilers fed the control diet also had the highest total monounsaturated fatty acids (MUFA) and n-6/n-3 compared to other diets. On the contrary, the total polyunsaturated fatty acids (PUFA), total omega 6 (n-6), total omega 3 (n-3) and the ratio of PUFA/SFA were lowest in broilers fed

Table 4.5. Effects of potassium humate and Aextra XAP dietary inclusions on fatty acid composition (%) of broiler chickens meat.

Fatty Acids	Dietary treatments					SEM
	Control	CM	CMenz	CMPh	CMenzPh	
Myristic	0.37 <sup>c</sup>	0.29 <sup>b</sup>	0.27 <sup>a</sup>	0.29 <sup>b</sup>	0.29 <sup>b</sup>	0.01
Myristoleic	0.07 <sup>c</sup>	0.03 <sup>b</sup>	0.02 <sup>ab</sup>	0.00 <sup>a</sup>	0.01 <sup>ab</sup>	0.01
Pentadecylic	0.08	0.08	0.07	0.09	0.08	0.01
Palmitic	23.86 <sup>c</sup>	21.08 <sup>a</sup>	21.18 <sup>ab</sup>	22.07 <sup>b</sup>	21.86 <sup>ab</sup>	0.24
Palmitoleic	4.65 <sup>c</sup>	2.58 <sup>b</sup>	2.35 <sup>b</sup>	1.80 <sup>a</sup>	2.46 <sup>b</sup>	0.24
Margaric	0.94	0.96	1.05	1.29	0.93	0.09
Heptadecenoic	0.06	0.14	0.19	0.09	0.14	0.07
Stearic acid	8.01 <sup>a</sup>	8.09 <sup>a</sup>	9.07 <sup>bc</sup>	9.84 <sup>c</sup>	8.39 <sup>b</sup>	0.42
Oleic	30.43 <sup>c</sup>	26.72 <sup>b</sup>	26.28 <sup>b</sup>	25.36 <sup>a</sup>	26.67 <sup>b</sup>	0.77
Vaccenic	4.54 <sup>b</sup>	4.39 <sup>b</sup>	4.39 <sup>b</sup>	4.07 <sup>a</sup>	4.27 <sup>b</sup>	0.09
Nonoadecanoic	0.47	0.48	0.53	0.66	0.46	0.05
Linoleic	19.33 <sup>a</sup>	25.54 <sup>c</sup>	24.27 <sup>c</sup>	22.98 <sup>b</sup>	24.96 <sup>c</sup>	0.65
Arachidic	0.04 <sup>b</sup>	0.05 <sup>c</sup>	0.04 <sup>b</sup>	0.04 <sup>b</sup>	0.03 <sup>a</sup>	0.01
γ-Linolenic	1.02 <sup>a</sup>	1.96 <sup>c</sup>	1.76 <sup>b</sup>	1.62 <sup>b</sup>	1.79 <sup>b</sup>	0.09
Heneicosanoic	0.24	0.25	0.27	0.33	0.23	0.03
Eicosadienoic	0.36 <sup>a</sup>	0.67 <sup>c</sup>	0.59 <sup>b</sup>	0.67 <sup>c</sup>	0.57 <sup>b</sup>	0.57
Eicosatrienoic	0.03 <sup>a</sup>	0.10 <sup>c</sup>	0.09 <sup>b</sup>	0.11 <sup>c</sup>	0.07 <sup>b</sup>	0.01
Erucic	0.83	0.76	0.75	0.84	0.70	0.07
Arachidonic	3.72 <sup>a</sup>	4.39 <sup>b</sup>	5.25 <sup>c</sup>	5.94 <sup>c</sup>	4.69 <sup>b</sup>	0.49
Eicosopentaenoic	0.20	0.22	0.19	0.26	0.20	0.03
Docosapentaenoic	0.32 <sup>a</sup>	0.63 <sup>b</sup>	0.69 <sup>b</sup>	0.81 <sup>c</sup>	0.62 <sup>b</sup>	0.06
Docosahexanoic	0.18 <sup>a</sup>	0.38 <sup>b</sup>	0.46 <sup>b</sup>	0.61 <sup>c</sup>	0.37 <sup>b</sup>	0.06

<sup>a, b, c</sup> Means in the same row with different superscripts are significantly different ( $P < 0.05$ ). Control (commercial broiler diet); CM (17.5 % CM inclusion); CMenz (17.5% CM inclusion + 0.3 g/kg Aextra XAP); CMPh (17.5% CM inclusion + 1.5% Potassium Humate, PH) and CMenzPh (17.5% CM inclusion + 1.5% PH + 0.3 g/kg Aextra XAP).

Table 4.6. The effect of potassium humate and Aextra XAP inclusions on fatty acids profiles (total nutritional indices) of broiler meat.

Parameter	Dietary treatments					SEM
	Control	CM	CMenz	CMPh	CMenzPh	
Total SFA	34.00 <sup>c</sup>	31.28 <sup>a</sup>	32.48 <sup>b</sup>	34.58 <sup>c</sup>	32.28 <sup>b</sup>	0.68
Total MUFA	40.61 <sup>c</sup>	34.61 <sup>b</sup>	33.99 <sup>ab</sup>	32.17 <sup>a</sup>	34.25 <sup>b</sup>	34.25
Total PUFA	25.39 <sup>a</sup>	34.09 <sup>c</sup>	33.53 <sup>b</sup>	33.25 <sup>b</sup>	33.47 <sup>b</sup>	0.62
Total n-6	23.52 <sup>a</sup>	30.68 <sup>c</sup>	30.23 <sup>c</sup>	29.73 <sup>b</sup>	30.33 <sup>c</sup>	0.58
Total n-3	1.88 <sup>a</sup>	3.42 <sup>c</sup>	3.29 <sup>bc</sup>	3.52 <sup>c</sup>	3.14 <sup>b</sup>	0.07
PUFA: SFA	0.75 <sup>a</sup>	1.09 <sup>c</sup>	1.03 <sup>b</sup>	0.96 <sup>ab</sup>	1.04 <sup>b</sup>	0.03
n-6/n-3	12.56 <sup>c</sup>	8.99 <sup>ab</sup>	9.22 <sup>b</sup>	8.47 <sup>a</sup>	9.68 <sup>b</sup>	0.21

<sup>a, b, c</sup> Means in the same row with different superscripts are significantly different ( $P < 0.05$ ). Total Saturated Fatty Acids =SFA; Total Mono Unsaturated Fatty Acids =MUFA; Total Poly Unsaturated Fatty Acids =PUFA; Total Omega- 6 Fatty Acids = n-6; Total Omega- 3 Fatty Acids = n-3; PUFA:SFA; n-6/n-3. Control (commercial broiler diet); CM (17.5 % canola meal inclusion); CMenz (17.5% CM inclusion + 0.3g/kg Aextra XAP); CMPh (17.5% CM inclusion + 1.5% Potassium Humate, PH) and CMenzPh (17.5% CM inclusion + 1.5% PH + 0.3g/kg Aextra XAP).

the control diet compared to other diets. The total n-3 FAs were highest in broilers fed CMPH diet followed by CM and CMenz diets respectively. For the n-6/n-3 ratio, the highest ratio was in broilers fed control whilst the lowest was obtained in those fed CMPH diet.

## **4.4 Discussion**

### *4.4.1 Carcass traits*

Results from the study showed a significant effect of diet on the breast muscle weight and breast muscle index. The breast muscle weight and the index are indicative of muscle mass in a carcass. The higher breast weights of broilers fed humic acid and enzyme complex diets could be attributed to the effect of the humic acid and an enzyme complex in stimulating effective digestion and muscle accretion. Li *et al.*, (2016) reported that the inclusion of humic acid in broiler diets can improve digestion dynamics and nutrient absorption ultimately regulating growth and change the metabolism to enhance animal carcass traits. Kocabagli *et al.* (2002) and Ozturk *et al.* (2012) also demonstrated a linear increase in body and carcass weights with the inclusion of HA substances in the broiler diet. On the other hand, Dalólio *et al.* (2015) also reported a significant improvement on breast performance at 42 days for broilers supplemented with enzyme complex as observed in the current study.

Diet, including other dynamic factors such as genetics, sex, slaughtering conditions and age of the animal can influence carcass traits (Young *et al.*, 2001; Havenstein *et al.*, 2003; Brickett *et al.*, 2007). Contrary to the current study, Khajali *et al.*, (2011) and Gopinger *et al.*, (2014) reported that broiler breast yield was reduced significantly when a diet of soybean meal was replaced with canola meal confirming that inclusion of enzymes can improve utilisation of canola meal in broiler diets. The observed lack of differences in all other traits in the current study is consistent with findings reported by Brandao *et al.*, (2007) where there was no effect

of diet on carcass characteristics on broilers fed different protein levels at the growth and finisher phases.

#### *4.4.2 Internal organs*

Changes in size and structure of internal organs can be indicative of the effect of diet and its components on the development and function of the organs. In the current study, although there were differences in sizes and weights of the internal organs, the differences were marginal. The results are consistent with findings by Janječić *et al.*, (2002) who observed that birds fed on diets containing 17% of rapeseed meal had increased organ weights. On the contrary, findings from other studies revealed lack of effect of diet on internal organs (Montazer-Sadegh *et al.*, 2008; Nagaraju *et al.*, 2014). Generally, an increase in liver and heart sizes could be indicative of the need to deal with toxic substance in feed. On the other hand, an increase in intestinal length could be indicative of the need to increase retention time for digestion processes (González-Alvarado *et al.*, 2008; Jiménez-Moreno *et al.*, 2010).

#### *4.4.3 Meat quality*

Meat pH is one of the most important factors that has an effect on other meat quality parameters. In the current study, diet had no effect on meat pH. Meat pH values obtained in the study were, however, higher than those reported for broilers by Li *et al.* (2016). Nevertheless, the pH values were within the 5.5 to 6.5 range that were reported for simple non-ruminants (Ao *et al.*, 2008). Due to post-mortem glycolytic activity that results in lactic acid accumulation in muscle, meat pH tends to decline significantly (Bai *et al.*, 2013). The pH value of meat can be reflective of drip loss and cooking loss whilst these two parameters consequently affect meat colour, water holding capacity and shear force values of fresh meat (Hofmann, 1994; Huff-Lonergan *et al.*, 2002).

The visual appearance of meat is one of the most vital meat quality attributes that influence acceptance of meat and meat products and purchasing decisions by consumers (Adeyemi and Sazili, 2014). The findings of the current study, show an increase in redness (Minolta a\*) with the inclusion of canola, Aextra XAP and Potassium humate in the diets. On the contrary broilers fed on the control and CMPH diets had lighter breast muscles. The results are consistent with findings by Ji *et al.* (2006) and Wang *et al.*, (2008) who observed that humic substance supplementation can improve the appearance of the meat, which is partly in agreement with the results of the current study. Although the precise underlying mechanism of the effect of humic substances is unknown, it appears that humic substances contains minute quantities of several minerals, including Fe, Mn and Cu which may influence changes in meat colour (Bai *et al.*, 2013)

Meat of broilers fed the control diet had the lowest water loss, which means it has the greatest capacity to retain water, whilst the meat of broilers supplemented with CMenz showed the least capacity to retain water. Similar observations were made in other studies (Pearce *et al.*, 2011; Hughes *et al.*, 2014) in which this meant that SBM had a great potential in contributing to quality meat compared to enzymes as they show lower WHC. Although diet had no effect on both drip loss and cooking loss, it has been observed that, generally, greater drip loss may induce a reduction in water-holding capacity and tenderness of meat (Wang *et al.*, 2012). The WHC is generally associated with the lipid peroxides content in the muscle (Macit *et al.*, 2003; Schaefer *et al.*, 1995). In the current study, the effect of diet on shear force values was insignificant. The shear force is an objective indicator of the toughness of the meat (Lonergan *et al.*, 2003). A lower shear force value is indicative of meat that is tenderer (Lonergan *et al.*, 2003; Li *et al.*, 2016).

#### 4.4.4 The proximate fat composition

The intra-muscular fat (IMF-marbling) content is an intrinsic indicator of meat quality (Yang *et al.*, 2009; Symeon, 2013). Our results show that IMF composition of breast muscle was not significantly affected for all the dietary treatments. This suggests that inclusion of CM, enzyme complex and humic acid salt in diets had no influence on FAs composition and biosynthesis during the regulation of IMF content (Cameron *et al.*, 2000).

#### 4.4.5 Fatty acid profiles and nutritional indices of broiler meat.

In the current study, the predominant fatty acids included the palmitic acid (C16:0), Oleic acid (C18:1c9), Linoleic acid (C18:2C9, 12, n-6) and stearic acid. This is consistent with findings from other studies (Laudadio and Tufarelli, 2010; Ahmed *et al.*, 2015). Broilers fed on the control diet had higher values for most of the saturated fatty acids (SFA), particularly myristic (C14:0), myristoleic (C14:1c9) and palmitic (C16:0) acids which have a great significance due to their hypercholesterolemic properties which are related with coronary heart disease (FAO/WHO, 2009; Ahmed *et al.*, 2015). On the contrary, the canola fed broilers had higher values for most polyunsaturated fatty acids (PUFAs) including, the linoleic (C18:2c9, 12 (n-6))  $\gamma$ Linolenic (C18:3c6, 9, 12 (n-3), eicosadienoic, eicosatrienoic, decosapentaenoic and decosahexaenoic acids. Interestingly, inclusion of humic acid salt in diets appeared to significantly increase the proportions of the PUFAs in broiler meat. Although the underlying mechanism is still not well understood, in pork, humic acid was observed to have an effect of increasing the fat marbling values and to reduce back fat thickness probably due its influence on protein and lipid distribution (Wang *et al.*, 2008).

The meat of broilers fed canola based diets were observed to be rich in PUFAs, total n-6 fatty acids and total n-3 fatty acids. This can be a result of the protein source and the other feed

additives in modulating the synthesis of intrinsic beneficial fatty acids that promotes the health of consumers. According to Grashorn, (2007), both n-3 and n-6 FAs in the meat play an immense role in human nutrition as they are originators of eicosanoids, leucotriens, and thromboxanes, which regulate the cardiovascular system and immunological processes (Ahmed *et al.*, 2015).

The PUFA: SFA and n-6/n-3 ratios are critical parameters used to evaluate the nutritional value of meat (Santos-Silva *et al.*, 2002; Ahmed *et al.*, 2015). Generally, meat with low PUFA: SFA ratio which is less than (0.4) and high n-6/n-3 ratio ( $> 5$ ) ratio may be considered to be poor and unfavourable since they might encourage an escalation in cholesterolaemia (Santos-Silva *et al.*, 2002). In the current study, inclusion of canola, enzyme complex and humic acid salt in diets resulted in an increase in PUFA/SFA ratio. Moreover, the inclusion of canola, enzyme complex and humic acid salt also reduced the n-6/n-3 ratio, particularly in the CMPh fed broilers although the values were above the recommended ratio of 5. Therefore, inclusion of canola, enzyme complex and humic acid salt resulted in a favourable effect of important FAs required for maintenance of human health.

#### **4.5 Conclusion**

From the results, it can be concluded that the inclusion of CM, Aextra XAP and potassium humate in broiler diets can have beneficial effects on the carcass and meat quality parameters in terms of breast weights, WHC and colour coordinates. Inclusion of CM, Aextra XAP and potassium humate in diets can also have the effect of increasing the proportion of PUFAs, n-6 and n-3 fatty acids and the PUFA/SFA ratio which are important in the assessment of nutritional value of meat. Therefore, inclusion of CM and natural feed additives could successfully improve the intrinsic meat quality properties of broilers for the benefit of

consumers. However, muscle deposition may have an impact on bone development and the ability of broilers to carry their own weight. Therefore, it may be important to assess the influence of humic acid and enzyme inclusion in diets on bone characteristics of broiler chickens.

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## CHAPTER 5

### EFFECTS OF HUMIC ACID AND ENZYMES INCLUSION ON BONE DEVELOPMENT, INTESTINAL HISTOMORPHOLOGY AND IMMUNE DEVELOPMENT IN BROILERS FED CANOLA-BASED DIETS

#### Abstract

The objective of the current study was to investigate the effect of dietary inclusion of humic acid and enzyme on bone development, histomorphology of internal organs and the incidence of rickets in broiler chickens fed canola-based diets. Two hundred and twenty broiler chickens were randomly allotted to 5 dietary treatments: the control (commercial broiler diet); CM (17.5% canola meal inclusion); CMenz (17.5% CM inclusion + 0.3g/kg Aextra XAP); CMPh (17.5% CM inclusion + 1.5% Potassium Humate, PH) and CMenzPh (17.5% CM inclusion + 1.5% PH + 0.3g/kg Aextra XAP). Each bird was individually placed into a plastic container previously filled with 3 cm water at 32°C. The time until each bird sat down as latency to lie down was recorded. For tibia gross lesion, growth plates were examined to assess prevalence of rickets visually. Leaner parameters of tibia, breaking bone strength and bone ash were also measured. Mineral content was obtained using an ICP Mass Spectrometer. The parameters measured for histomorphology included the height and width of the intestinal villi as well as the widths of the crypts. Diet had an effect on latency to lie test with broilers in CMenz having the highest tendency to lie ( $2.88 \pm 2.69$  min). The highest standing persistency was observed in CMenzPh ( $11.19 \pm 2.69$  min) chickens followed by those offered the control diet ( $9.05 \pm 2.69$  min). However, diet had no effect on tibia biomechanics. On the contrary, the dietary treatments had an influence ( $P < 0.05$ ) on the macro mineral content (calcium, phosphorus, magnesium and potassium) apart from sodium. Intestinal morphometric parameters demonstrated some differences in the height and width of the intestinal villi and intestinal crypts. Gross lesion

analysis showed high prevalence of rickets in CMenz chickens, whilst the inclusion of canola and PH appeared to improve distribution and density of lymphoid tissue in the peripheral and central follicles building tissues of the bursa of Fabricius and thymus which are major organs of immune defence in chicken.

## **5.1 Introduction**

In broiler chickens (meat-type) selection for rapid growth over a short production cycle has inadvertently resulted in high incidences of immune deficiency and bone disorders such as tibia dyschondroplasia, rickets and associated valgus-varus deformities leading to lameness (Flemming, 2008; Dinev, 2012). Bone conditions related to weakness of legs have been identified as a severe problem in broiler chickens that grow fast, resulting in reduced profits due to low productive efficiency as well as raising concerns about the welfare of the chickens (Ruiz-Feria *et al.*, 2014). The rapid growth over a short production cycle has been achieved through use of commercial growth promotants. Due to emergence of microbial resistance to antibiotics as well as persistence of drug residues in meat and meat products, a ban on the use of commercial growth promotants was effected in most countries beginning with the European Commission (EC Regulation No. 1831/2003). The ban encouraged exploration of utility of non-therapeutic alternatives including organic acids, enzymes, probiotics and nutrient sources that contain secondary plant metabolic elements with an influence on the general health of chickens (Lückstädt and Mellor, 2011; Khan and Iqbal, 2015).

Organic acids, including humic acids, enzymes and secondary plant metabolites have variable physical and chemical properties that allows them to influence gastronomic functions of feed in poultry. In Chapter 4, the inclusion of humic acid and enzymes positively influenced muscle deposition, which is associated with bone development and the ability of broilers to carry their

own weight. Inclusion of exogenous enzymes in diets may ensure effective release and assimilation of nutrients and minerals in the gut, stimulating efficient nutrient utilization and more importantly, active bone growth (Scholtz *et al.*, 2007; Emami *et al.*, 2013). On the other hand, humic acids may induce intestinal morphological alterations, stimulate changes in intracellular divalent calcium levels and act as dilators increasing the mucosal and cellular permeability (Pizzari *et al.*, 2000; Johnsson *et al.*, 2015). The increased permeability allows easier assimilation of minerals from the gut and transfer from blood to the bone and cells resulting in effective bone development (Stepchenko *et al.*, 1991; Dinev. 2012a). Like other organic acids, humic acid may also play a significant role in the development of immune organs particularly the bursa of Fabricius and thymus, which are the major components of the avian immune system (Abdel-Fattah *et al.*, 2008; Ghazala *et al.*, 2011).

Studies have been conducted on the effect of enzymes and humic acids salts on nutrient utilization and growth performance in poultry and some animal species (Islam *et al.*, 2005; Arif *et al.*, 2016). However, information on the influence of enzymes and humic acid salts inclusion in low-cost diets on organ and bone development in broilers is scarce. This study was, therefore, conducted to determine the influence of potassium humate and exogenous enzyme inclusion on intestinal histomorphology, bone and immune development, and incidents of rickets in broiler chickens fed canola-based diets.

## **5.2 Material and Methods**

### *5.2.1 Study site, source of diets, animal management*

The description of the study area is explained in Section 3.2.1-3.2.3 whilst the Section 3.2.4 explains the experimental design and dietary treatments.

### *5.2.2 Latency to Lie*

At day 35, 4 broiler chickens per treatment were exposed to the latency to lie (LTL) test, as described by Berg and Sanotra (2003). This test is established on the fact that body interaction with water is a novel experience for broiler chickens. In brief each bird was individually placed into a plastic container previously filled with 3 cm water at 32°C. The time until each bird sits down was recorded, according to the principle that the better leg health the broilers has, the longer it will stand up to avoid body contact with the water. If the chicken was still standing after 600 seconds which is equivalent to 10 minutes, the test was interrupted, which means the legs are strong and healthy.

### *5.2.3 Slaughter procedures*

The description of the slaughter procedure is explained in section 4.2.2

### *5.2.4 Tibia gross lesion analysis*

Tibia gross anatomy was conducted to assess the prevalence of rickets in broilers from different dietary treatments. The muscles around the tibia were carefully removed then the proximal end was dissected with very sharp scalpel to expose the epiphyseal plate. The growth plates of the tibia bones were examined visually for gross lesions.

### *5.2.5 Tibia linear parameters and bone breaking strength*

At necropsy on day 42 days, 6 right tibiae from each treatment were surgically removed, defleshed and cleaned of all tissue, including cartilage caps (periosteum) by hand, and weighed to obtain the tibia weight (TW). Tibia diameter proximal end (TDPE), tibia width diameter (TWD), tibia length diameter (TLD) and tibia diameter distal end (TDDE) were determined using a Toshiba-Rotanode x-ray (Toshiba Electron Tubes and Devices, Tochigi, Japan)

connected to the Point-of-Care (CR140) system for digital imaging. After linear measurement, each of the tibiae was placed on an adjustable 3-point bend/ snap fixture fitted on a heavy-duty TA. XT platform of a Texture Analyzer (model TA-XT plus, Stable Microsystems, Surrey, UK) and broken with a 6-cm flat head probe attached to a 50-kg load cell reporting the breaking force in Newtons. The distance between the bone supports was 50 mm. The breaking bone strength (BBS) was recorded as the peak load before the bone breakage.

#### *5.2.6 Bone ash and mineral content*

After the measurement of breaking strength, the tibia pieces were collected, defatted and ashed. The samples were placed into a muffle furnace at 600°C for 24 hours and cooled down in a desiccator before being weighed (final) to determine ash percentage. Tibia bone ash was expressed as a percentage as follows:

$$\text{Tibia bone ash \%} = \frac{(\text{Tibia ash weight})}{(\text{Tibia dry weight})} \times 100$$

The mineral content was analysed in the Animal Health Laboratory (NWU) using the dry ashing macro and trace minerals methods and following the guidelines of the Agri-Laboratory Association of Southern Africa (AgriLASA, 1998). The ash was weighed and digested with 1 mL of 55% nitric acid overnight. After 24 hours, samples were then gently transferred to McCartney bottles without disturbing the sediment. The concentrations of Ca, P, Mg, Na and K were then determined using an ICP Mass Spectrometer (Perkin-Elmer, 1982, NexION 300Q). Macro-minerals were presented as mg/l.

#### *5.2.7 Histomorphology of internal organs*

At necropsy, based on random sampling from the 5 dietary treatments, 6 tissue samples per treatment were obtained for histological examination of the bursae of Fabricius, thymi and

sections of the small intestines (duodenum, jejunum and ileum) and the caecae. The obtained tissue samples were thoroughly washing with chilled phosphate buffer (PBS, pH – 7.2). Intestinal segments measuring 1 cm in length, were then dissected and fixed on cork lamellae. After collecting the samples they were washed again with PBS buffer to eliminate intestinal contents and then they were placed in cold 10% buffered and neutralized formalin. Thereafter the samples were embedded in paraffin and then micro samples with a thickness of approximately 4 µm were obtained and stained with hematoxylin and eosin (H / E). The various intestinal morphometric measurements were then determined using a light microscope (OPTIKA B - 500 TIFL, software OPTIKA VISION PRO - Opticam pro plus 519CU - 5D95B401-8C000010). The parameters measured included the height and width of the intestinal villis and width of the crypts.

### *5.2.8 Statistical analysis*

Data on LTL test, intestinal morphometric measures and tibia parameters was analysed using GLM procedure of SAS (2010). The statistical model was as follows:

$$Y_{ijk} = \mu + D_i + \varepsilon_{ijk}$$

Where:  $Y_{ijk}$ = response variables,  $\mu$  = population mean,  $D_i$ = effect diets and  $\varepsilon_{ijk}$  = random error assumed to be normally and independently distributed. For all statistical tests, significance was declared at  $P < 0.05$ . Where significant difference was detected mean separation was done using the PDIFF option of SAS (2010).

## **5.3 Results**

### *5.3.1 Latency to lie test*

Diet had a significant ( $P < 0.05$ ) effect on latency to lie down test. Broilers in CMenz had the highest tendency to lie as reflected by the lowest time before they lay down ( $2.88 \pm 2.69$

minutes). The highest standing persistency was observed in CMenzPh ( $11.19 \pm 2.69$  minutes) followed by the control ( $9.05 \pm 2.69$  minutes) and CM ( $6.49 \pm 2.69$  minutes).

### *5.3.2 Tibia gross lesion analysis*

Results on the gross lesion analysis and incidence of rickets are shown in Figures 5.1 to Figure 5.5. Broiler chickens in the control had a mix of both long and short tibiae, where longer tibia bones indicated the normal bones, whilst the short tibia bone indicated the presence of rickets in bones. Moreover, the white stroma of the tibia bone from broilers in the control were enlarged compared to the normal bone. The proximal metaphyseal region of the bones also varied with differences of between 2 and 3 mm. Furthermore, the difference in the redness of the tibia bone marrow was not that distinguishable, showing a lack of a clear transition between the hypertrophic and proliferative zones. The tibia bones of broilers fed CM showed abnormal conversion of organic material in the bone mass with the white stroma revealing a transition of the proliferative zone of the growth plate into curve shaped projection. This observation can be due to disproportionate amounts of calcium and phosphorus in the diet. The tibiae from broilers in CMenz, CMPH and CMenzPh showed gross lesions associated with rickets manifested by the enlargement of the proximal metaphysis. The proliferative region also showed a curved deformation and the transition between the hypertrophic and proliferative zones was irregular. This was a clear sign of leg weakness, which could cause fracture of the bones. Broilers in CMenz showed more signs of bone abnormalities compared to all other treatments.



Figure 5.1. Significant widening in the region of the proximal metaphyseal tibiotarsus in broilers offered the control diet, which indicates rickets (right). Left – normal bone.

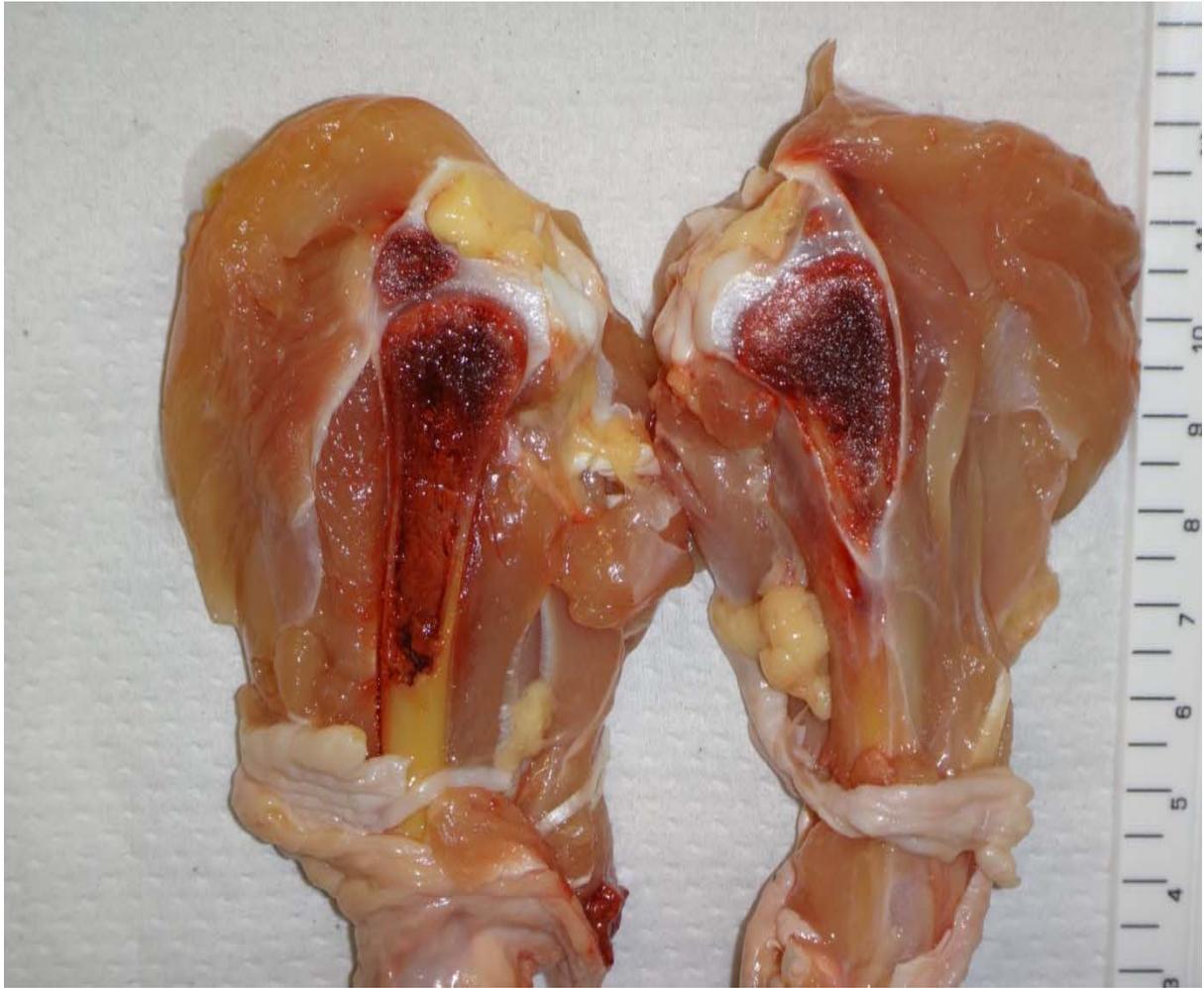


Figure 5.2. Thickening and deformation in the region of the proximal metaphyseal tibiotarsus in broilers offered canola meal inclusion diet indicating rickets (right). Left – normal bone.



Figure 5.3. Left – normal bone. Widening (in the middle) and deformation (on the right) in the region of the proximal metaphyseal tibiotarsus indicating rickets in broilers offered 17.5% CM inclusion + 0.3g/kg Aextra XAP diet.



Figure 5.4. Widening (left) and deformation (right) in the region of the proximal metaphyseal tibiotarsus indicating rickets in broilers fed 17.5% CM inclusion + 1.5% Potassium Humate diet.



Figure 5. 5. Widening and minor deformation (left) in the region of the proximal metaphyseal tibiotarsus indicating rickets in broilers fed 17.5% CM inclusion + 1.5% PH + 0.3 g/kg Aextra XAP: Right – relatively normal appearance of the same region of the bone.

### 5.3.3 *Tibia biomechanical parameters*

The effects of potassium humate and enzyme complex inclusion in canola meal diets on tibia bone parameters are presented in Table 5.1. Diet had no effect on TW, TDPE, TLD, TWD, TDDE and BBS. Nevertheless, regardless of lack of variation among treatments, a general linear increase in tibia measurements was observed with inclusion of canola and the different feed additives in diets.

### 5.3.4 *Tibia bone mineralisation*

The effects of potassium humate and Aextra XAP inclusion in canola-meal-based diets on tibia bone ash and macro mineral content are presented in Table 5.2. Diet had no influence on the bone ash ( $P < 0.05$ ). On the contrary, the diet had an influence ( $P < 0.05$ ) on the macro mineral content apart from sodium (Na). Broilers in CMPH (15064.02 mg/l) had high levels of Ca, followed by the control (14960.44 mg/l). CMenzPh (6579.65 mg/l) had the lowest Ca levels. Phosphorus (P) content was however higher in the control (1749.46 mg/l) chickens than all other treatments. With regards to the Ca: P ratios, bone mineral of broilers in the control (8.509) and CM (8.028) had the highest ratios whilst CMenzPh (4.991) had the lowest ratio out of all treatments. The magnesium (Mg) content in CMPH chickens (151.52 mg/l) was the highest whilst CMenzPh (64.35 mg/l) was the lowest. Potassium (K) content in CMPH (570.97 mg/l); CMenzPh (511.64 mg/l) and CMenz (244.23 mg/l) were the lowest compared to the control and CM (438.41; 450.60 mg/l).

Table 5. 1. The effect of humic acid and enzyme complex inclusions on tibia biomechanics of broilers fed canola-based diets.

Parameters	Dietary treatments					SEM
	Control	CM	CMENZ	CMPH	CMenzPH	
Tibia weight (g)	11.38	13.45	12.67	12.15	14.13	0.89
Tibia diameter proximal end (cm)	2.29	2.19	2.32	2.25	2.37	0.10
Tibia width diameter (cm)	0.77	0.93	0.78	0.79	0.80	0.40
Tibia length diameter (cm)	9.77	10.19	9.75	9.98	10.12	0.15
Tibia diameter distal end (cm)	1.79	1.95	1.73	1.69	1.81	0.08
Bone breaking strength (kg)	33.96	28.68	31.99	35.19	36.57	2.13
Latency to lie test (min)	9.05 <sup>ab</sup>	6.49 <sup>b</sup>	2.88 <sup>c</sup>	8.63 <sup>b</sup>	11.19 <sup>a</sup>	2.69

<sup>a, b, c</sup>Means in the same row with different superscripts are significantly different ( $P < 0.05$ ). Control (commercial broiler diet); CM (17.5 % canola meal inclusion); CMenz (17.5% CM inclusion + 0.3 g/kg Axtra XAP); CMPH (17.5% CM inclusion + 1.5% Potassium Humate, PH) and CMenzPh (17.5% CM inclusion + 1.5% PH + 0.3 g/kg Axtra XAP).

Table 5. 2. Tibia ash and macro mineral content of broiler tibia bone fed dietary treatments

Parameters	Dietary treatments					SEM
	Control	CM	CMenz	CMPh	CMenzPh	
Tibia ash (%)	41.73	37.58	38.54	35.44	39.38	3.99
Calcium (mg/l)	14960.44 <sup>b</sup>	11765.35 <sup>c</sup>	11895.04 <sup>c</sup>	15064.08 <sup>b</sup>	6579.65 <sup>a</sup>	1385.66
Phosphorus (mg/l)	1749.46 <sup>c</sup>	1466.20 <sup>ac</sup>	1621.34 <sup>c</sup>	2360.42 <sup>b</sup>	1277.70 <sup>a</sup>	174.73
Magnesium (mg/l)	141.54 <sup>bc</sup>	116.11 <sup>c</sup>	116.11 <sup>c</sup>	151.52 <sup>b</sup>	64.35 <sup>a</sup>	13.55
Sodium (mg/l)	195.68	170.53	216.19	208.29	117.18	27.19
Potassium (mg/l)	438.41 <sup>bc</sup>	450.60 <sup>bc</sup>	244.23 <sup>a</sup>	570.97 <sup>b</sup>	511.64 <sup>b</sup>	58.31
Ca: P	8.509 <sup>b</sup>	8.028 <sup>b</sup>	7.373 <sup>bc</sup>	6.362 <sup>c</sup>	4.991 <sup>a</sup>	0.211

<sup>a,b,c</sup> Means in the same row with different superscripts are significantly different ( $P < 0.05$ ). Ca: P = Calcium phosphorus ratio. Control (commercial broiler diet); CM (17.5 % canola meal inclusion); CMenz (17.5% CM inclusion + 0.3 g/kg Aextra XAP); CMPh (17.5% CM inclusion + 1.5% Potassium Humate, PH) and CMenzPh (17.5% CM inclusion + 1.5% PH + 0.3 g/kg Aextra XAP).

### 5.3.5 Serum Ca and P levels

Table 5.3 shows the effects of dietary treatments on mineral content of serum calcium and phosphorus of broilers. There was no difference observed on Ca levels across treatments. However, diet significantly ( $P < 0.05$ ) affected serum P levels. Serum P increased with inclusion of canola and the different feed additives. Phosphorus levels were higher in CMPh and CMenzPh chickens whilst the control chickens have the lowest P levels.

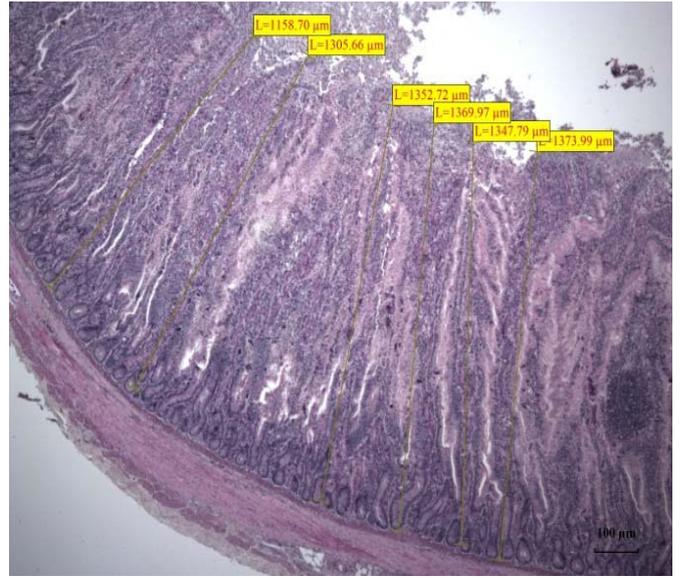
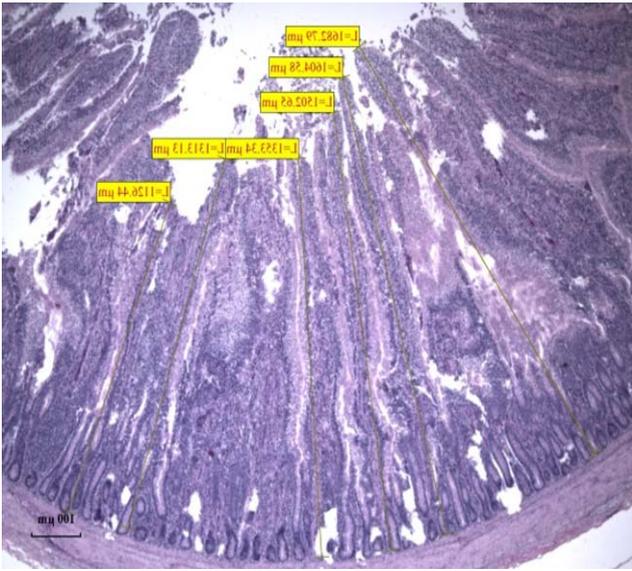
### 5.4.6 Histomorphology of internal organs

Microscopic analysis of the intestinal tract of the broilers showed desquamative processes in mild to moderate degree, affecting the apical part of the intestinal villi, prevailing in broilers from the control and CMenzPh groups. However, in all dietary treatments trace to mild manifestation of mucosal hyperemia and sub-mucous edema was observed. The results on intestinal morphometric parameters demonstrated some differences in the height and width of the intestinal villi and in the width of the intestinal crypts (Figures 5.6 and Figure 5.7 as well as Table 5.4). The values of these three parameters showed some significant variations ( $P < 0.05$ ) among the five dietary treatments. The widest and tallest intestinal villi were observed in broilers in CM, CMenz and CMPh, whilst the widest crypts were observed in CM (Table 5.4). Examination of organs of the central immune system (thymi and bursae of Fabricius) revealed some variation in the density of lymphoid tissue build-up in their structural elements (Figure 5.8 and 5.9). In CM and CMenz, well established, uniformly distributed and very well-defined density of lymphoid tissue in the peripheral and central follicles building parenchyma organs were observed. Nevertheless, chickens in the control had some reduction in thymic volume units and the follicles of the bursa of Fabricius, both in the cortical and the core regions. The central follicles in these cases were characterized by poorly pronounced density of lymphoid tissue (light area).

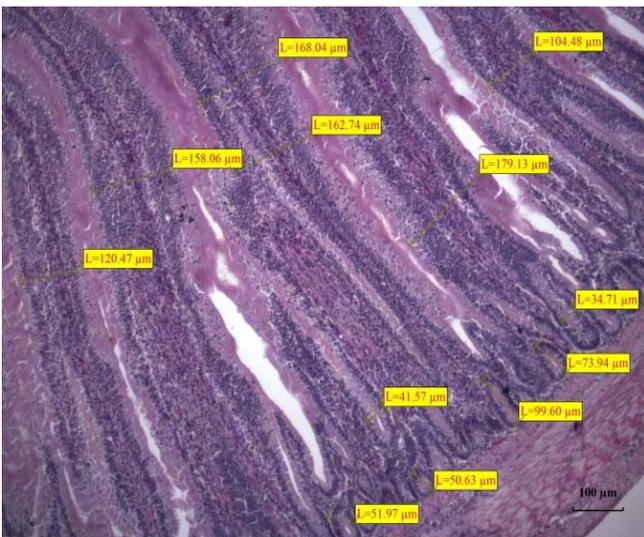
Table 5. 3. The effect of enzymes and humic acid inclusions on serum Ca and P levels in broilers fed canola based diets

Parameters	Dietary treatments				
	Control	CM	CMenz	CMPh	CMenzPh
Calcium (mg/l)	2.52 ± 0.06	2.49 ± 0.07	2.58 ± 0.07	2.54 ± 0.07	2.44 ± 0.81
Phosphorus (mg/l)	0.29 ± 0.01 <sup>a</sup>	0.31 ± 0.02 <sup>a</sup>	0.35 ± 0.01 <sup>ab</sup>	0.4 ± 0.03 <sup>b</sup>	0.49 <sup>b</sup> ± 0.01

<sup>a, b</sup> Means in the same row with different superscripts are significantly different ( $P < 0.05$ ). Control (commercial broiler diet); CM (17.5 % canola meal inclusion); CMenz (17.5% CM inclusion + 0.3 g/kg Axtra XAP); CMPh (17.5% CM inclusion + 1.5% Potassium Humate, PH) and CMenzPh (17.5% CM inclusion + 1.5% PH + 0.3 g/kg Axtra XAP).



a) Height of intestinal villas: duodenum (Control – 1)      b) Height of intestinal villas: Jejunum (CM – 3)



c) Width of villas and crypts: Ileum (CMPH – 2)      d) Width of villas and crypts. Jejunum (CMenz – 3)

Figure 5. 6. Intestinal morphometric measurements (height and width of villi and crypts) for broilers in different treatments (H/E, Bar=100μm).



Figure 5. 7. Morphometric measurements of the width of villas and crypts. Caeca (CMenzPh – 3), H/E, Bar=100µm.

Table 5. 4. The effect of enzymes and humic acid inclusions on intestinal morphometric measurements in broilers fed canola based diets  
Bar=100µm.

Morphometric (µm)	Dietary treatments					SEM
	Control	CM	CMenz	CMPh	CMenzPh	
Villus height	511.05	1308.46	565	687.29	300.47	51.31
Villus width	148.35	150.23	48.27	176.98	106.88	26.32
Crypts depth	23.55	73.01	46.03	46	39.48	3.25

<sup>a,b,c</sup> Means in the same row with different superscripts are significantly different ( $P < 0.05$ ). Ca: P = Calcium phosphorus ratio. Control (commercial broiler diet); CM (17.5 % canola meal inclusion); CMenz (17.5% CM inclusion + 0.3g/kg Aextra XAP); CMPh (17.5% CM inclusion + 1.5% PH) and CMenzPh (17.5% CM inclusion + 1.5% PH + 0.3g/kg Aextra XAP).

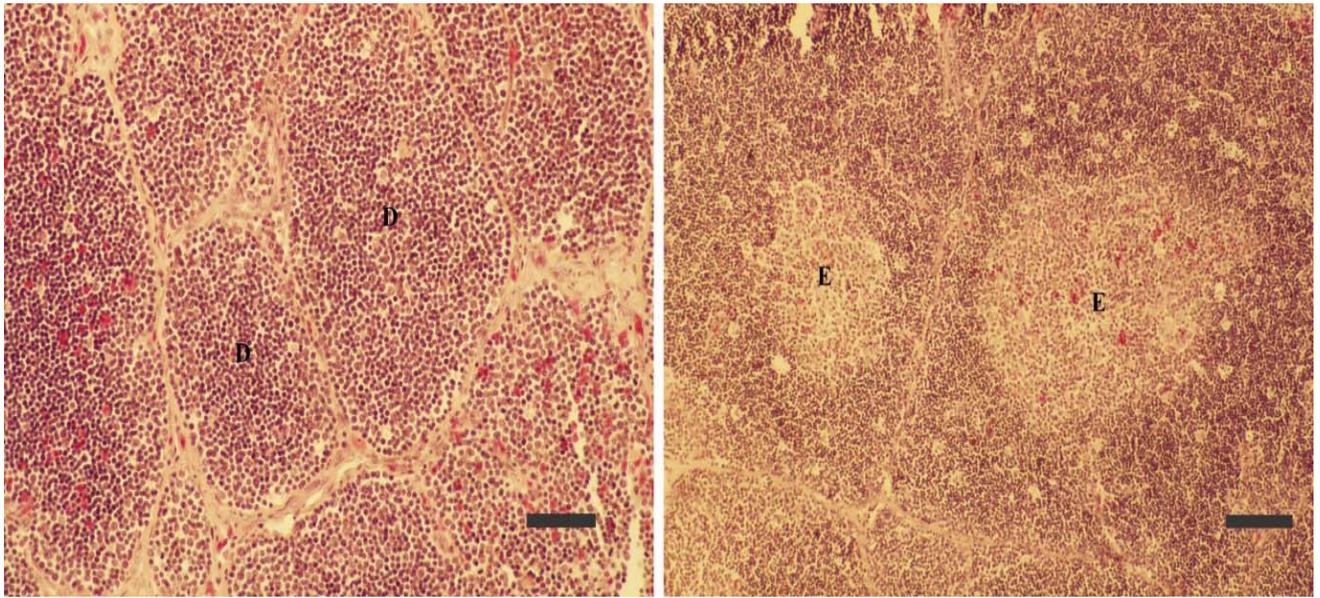


Figure 5.8. **Left panel:** Thymus in chicken in CM. Histologically established uniformly distributed, very well expressed density of lymphoid tissue in the peripheral and central part of the follicles (D), building the parenchyma of the organ, H/E, bar=40  $\mu\text{m}$ . **Right panel:** Section of the thymus in chickens in control. There is some reduction in the volume of thymic units, both in cortical and in the core part. Slight density of lymphoid tissue in the central part of follicles (light area - E), H/E, bar=40  $\mu\text{m}$ .

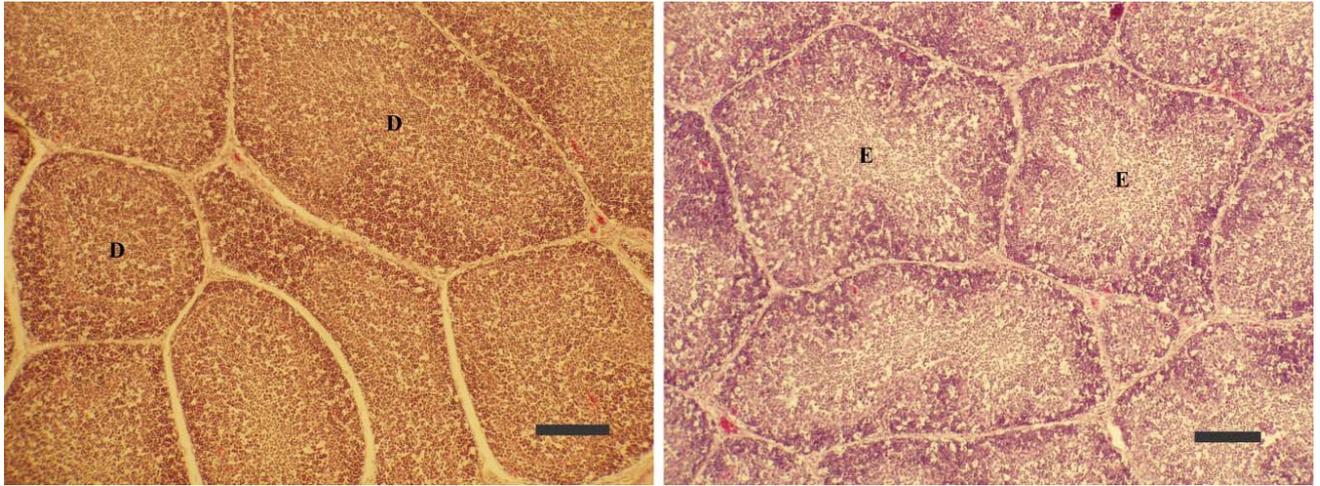


Figure 5. 9. **Left panel**: Bursa of Fabricius, cut surface, in CM. Functional lymphoid follicles with active central part of the lymphoid tissue (dense area - D), occupied mucosal folds, H/E, bar=30  $\mu$ m. **Right panel**: Bursa of Fabricius, cut surface, in control. Uniformly distributed lobules in the mucosal folds. Relatively underactive lymphoid tissue in the central part (pale zone - E) of the follicles X H/E, bar = 30  $\mu$ m.

## 5.4 Discussion

### 5.4.1 Latency to lie

Latency to lie (LTL) test as described by Weeks *et al.*, (2002) is a prominent method that was developed to indirectly measure bone development and bone strength in broilers. Water as an aversive stimulus has been shown to provide avoidance motivation resulting in the broilers with strong bones to stand as long as possible whereas those with poor bone development may easily succumb to their weight and sit down sooner (Berg and Sanotra, 2003). In the current study, broilers that had humic acid in their diets had the highest standing persistency whilst those fed a diet with enzyme complex only had the weakest bones. The longer standing persistency in broilers fed diets with humic acid could be a result of the high amounts of minerals in canola diets coupled with the actions of the humic acid in stimulating mineral assimilation dynamics in the gut and transfer from blood to the bone and cells allowing for development of stronger bones (Stepchenko *et al.*, 1991).

### 5.4.2 Tibia gross lesions

Generally, gross lesion analysis of the tibiae revealed clear evidence of subclinical rickets in broilers from all treatments although CMenz was more affected. This was attributed to the disproportionate amount of Ca and P in the diets. However, it could not be established in the current study whether the rickets were hypocalcaemic or hypophosphataemic. As observed in other studies, the type of rickets could not be determined due to the insufficient intensity of lesion occurrence in the bones (Dinev, 2009, 2011). Rickets are normally manifested as a result of dietary calcium (Ca), phosphorus (P) and vitamin D3 deficiency, impaired Ca/P ratio or impaired absorption of one or both of these macro-elements (Dinev., 2011). Erroneous feed mixing has also been shown to provoke outbreaks (Riddell, 1992). In both forms (hypocalcaemic or hypophosphataemic), the proper calcification of cartilage is impaired with

deficiency. In the current study, it appears that the lesions were not severe enough to cause lameness in all treatments including CMenz which had the highest incidences of subclinical rickets. The observed transition of proliferative zone of the growth plate into wedge-shaped projections in most of the tibiae was consistent with observations on broilers affected by rickets, as reported in other studies (Dinev, 2012b). The age when the lesions are usually prevalent in chickens is around the age of 35 days. This period is associated with intensive muscle growth and hence, cumulative weight may perhaps also be used to predict lesions (Dinev, 2012a). Moreover, mineralization during this period may affect the bone strength in which it allows the skeleton to resist the gravity and additional loading (Boivin and Meunier, 2002).

#### *5.4.3 Tibia bone parameters and bone mineralization*

Although no differences were observed with regards to tibia bone parameters, a marginal increase in these measures was observed with inclusion of canola meal and PH in the diets. Tibia biomechanical characteristics such as bone breaking strength, bone density, bone mineral content and bone ash (Onyango *et al.*, 2003; Kim *et al.*, 2006; Shim *et al.*, 2008) are usually used as indicators of mineral adequacy in broiler diets. In the current study, the marginal increase in the parameters could be due to the increased availability of Ca and P provided by canola in diets. Moreover, this could be attributed to the ability of humic acid to increase the degree of mineralization of the bone matrix (Stepchenko *et al.*, 1991). Generally, a lack of specific mineral such as Ca and P in the diets can result in poor bone development (Selle *et al.*, 2007). Inclusively, the results of the current study suggest that bone breaking strength, bone density, bone mineral content and bone ash may be sensitive indicators of dietary Ca and P levels in broiler chickens (Hall *et al.*, 2003; Venäläinen *et al.*, 2006). Generally, the macro mineral Ca has comparatively higher accessibility for most poultry feedstuffs, however the

accessibility of P differs largely depending on the source (Williams *et al.*, 2000; Waldenstedt, 2006). That is the reason why a lack of P does not convey any response to phytase supplementation when Ca is also limiting (Yan *et al.*, 2006). Furthermore, according to Talaty *et al.*, (2009) insufficient dietary sources of Ca can result in blood hypocalcaemia, which may lead to reduced bone strength and mineralization. Calcium and phosphorus have an intrinsic role in the structure and metabolism of bone. Phosphorus also plays an intrinsic role in energy accumulation.

#### *5.4.4 Histomorphology of internal organs*

Good gut and intestinal health in broiler production is critical for optimisation of feed utilisation efficiency and ultimately growth rates. In the current study, the inclusion of canola meal and PH had minimal effect on gut mucosa as highlighted by trace manifestation of mucosal hyperemia and submucosal edema, which was consistent with all dietary treatments. Nevertheless, the inclusion of canola and PH in diets significantly increased the intestinal morphometric parameters of the broilers. Generally, PH inclusion in diets have been observed to induce intestinal morphological modifications, increasing the mucosal and cellular permeability (Stepchenko *et al.*, 1991). The results of the study are consistent with findings from other studies which demonstrated an increase in villus height and crypt depth with inclusion of organic acids in broiler diets (Kum *et al.*, 2010; Rodríguez-Lecompte *et al.*, 2012). The trophic effect of the organic acids such as humic acid could be significant in the stimulation of proliferation of normal crypt cells, enhancing healthy tissue turnover and maintenance (Leeson *et al.*, 2005; Panda *et al.*, 2009). Moreover, the presence of humic acid and secondary plant metabolites in canola may contribute to reducing the bacterial colonization of the intestinal mucosa and facilitate increased efficiency of metabolic processes as observed in other studies (Khan, 2013).

The bursa of Fabricius and thymus are central lymphoid tissues, peculiar to chickens, which are involved in immune response. Whilst some variation in the density of lymphoid tissue build-up in their structural elements was observed among the treatments, the inclusion of canola and humic acid in diets appeared to result in an improvement in the distribution and density of lymphoid tissue in the peripheral and central follicles building parenchyma organs in CM to CMenzPh diets. This is significant in enhancing immune response in boiler chickens. Several studies have demonstrated the importance of organic acids such as humic acid in immune response. Abdel-Fattah *et al.* (2008); Ghazala *et al.* (2011) and Houshmand *et al.* (2012) observed that birds fed an organic acid-supplemented diet had heavier immune organs (bursa of Fabricius and the thymus) and also a higher level of globulin in their serum which is an indicator for measuring immune response. Although broiler chickens in the control had some reduction in thymic volume units and the follicles of the bursa of Fabricius, this was not a manifestation of lymphocytic depletion of the thymus and bursa of Fabricius.

## **5.5 Conclusions**

The results from the study showed that inclusion of PH in diets can positively influence bone and immune system development. Inclusion of enzyme alone resulted in broilers with lowest standing persistence and high prevalence of bone abnormalities associated with rickets. Inclusion of PH and enzymes stimulated growth of intestinal villi. Canola meal and PH inclusion in diets was also shown to improve distribution and density of lymphoid tissue in the peripheral and central follicles building tissues of the bursae of Fabricius and Thymus as such had a positive influence on the immune development. The findings from the study therefore validate the use and efficiency of potassium humate and canola meal as potential agents for bone and immune system development in broiler chickens.

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## CHAPTER 6

### 6 GENERAL DISCUSSION AND CONCLUSIONS

#### 6.1 General discussion

The concept of using natural humic acid such as potassium humate and enzyme supplementation as an alternative feed additive in animal nutrition has gained increasing importance, particularly after the ban on antibiotic use in feeds as growth promoters (Ceylan *et al.*, 2003; Karaoglu *et al.*, 2004). The research study had the main objective to enhance canola-based diets using Aextra XAP and potassium humate. It was observed in the results that there were no significant differences on ADFI across all treatments for both grower and finisher phases. This may be due to competition among broilers as they are meat type chicken to convert feed to meat. Interestingly the CM had higher ADG compared to birds on all the other diets. However, the highest FCR was observed in broilers offered the control diet whilst the lowest value was observed in CM broilers. Cumulative weight gain of birds fed diet CMenzPh was the highest throughout the experimental period. In the grower phase, The PC was highest in CMenzPh followed by CMenz. However, CM and CMPH had the highest PER whilst the control had the lowest. Specific growth rate was also higher in CM than all other treatments. In all instances, the control had the lowest values for PC, PER, SGR and GE in the grower phase. Growth performance indices were improved in the canola-based diets probably due to the effects of growth promoting agents (humic acid and enzymes) as well as secondary plant metabolites in canola in improving feed utilisation efficiency. Haematological parameters were not influenced by dietary treatments however, serum biochemical indices such as AST and sodium content, were significantly influenced by dietary treatments but not ALP, ALT, total protein, potassium, albumin, total calcium, cholesterol and magnesium. The results therefore suggest that inclusion of canola meal at 17.5% can not cause any barriers to the protein digestion dynamics and utilisation by broiler chickens. Therefore, canola can be used to replace

soybean meal at that level without major effects on blood metabolites. Diet had no effect on all carcass traits apart from breast muscle weight and breast muscle index. The results on meat quality measurements also showed a lack of significant effect of diet on pH and temperature measurements, drip loss and shear force values of the breast muscle. However, diet had a significant effect on the 3 meat colour coordinates and WHC. With regards to meat colour, broiler muscle in the control and CMPh groups had the highest values for L\* whilst the meat from broilers fed CMenzPh had the lowest. The results of WHC showed that breast muscles from broilers fed the control diet with the lowest value had the greatest WHC. With regards to fatty acid profiles, higher values for PUFAs, n-3 fatty acids and n-6 fatty acids were observed in the CM containing diets particularly the CMPh group. The inclusion of CM, enzymes and humic acid salt increased the PUFA/SFA ration whilst at the same time reducing the n-6/n-3 ratios which are important in assessing the nutritional value of the meat. The results observed suggested that inclusion of CM and natural feed additives could effectively improve the intrinsic meat quality properties of broilers for the benefit of consumers and how muscle deposition may have an impact on bone development and the ability of broilers to carry their own weight. Diet had an effect on the latency to lie test with broilers in CMenz having the highest tendency to lie. The highest standing persistency was observed in CMenzPh followed by the control group which implied that the two diets led to the optimum bone strength qualities. Dietary treatments had an influence on the macro mineral content apart from sodium. Intestinal morphometric parameters demonstrated some differences in the height and width of the intestinal villi and in the width of the intestinal crypts. Gross lesions analysis showed high prevalence of rickets in CMenz, whilst the inclusion of canola and PH appeared to improve distribution and density of lymphoid tissue in the peripheral and central follicles building tissues of the Bursa of Fabricius and the thymus. Overall, canola meal was shown to have great potential as an alternative of soybean meal in broiler diets. Collectively, findings from the study

can be helpful in designing low-cost feed formulations that will of benefit to consumers and improve growth performance, health status, carcass characteristics, meat quality and bone development in poultry farming systems in future.

## **6.2 Recommendations**

- ✓ Farmers and feed companies can replace soybean meal in the diets of broilers with canola meal at levels up to 17 % to improve the performance of the animals.
- ✓ Inclusion of a complex of enzymes in canola diets can improve utilisation of the protein sources although caution has to be taken due to some effects on bone development.
- ✓ Humic acids can be included in diets at levels up to 1.5% with significant improvement in meat quality, bone development and immune development in broilers.
- ✓ Cost benefit analysis

### **6.3 References**

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**CHAPTER 7**

**APPENDICES**

**Appendix 1. 1. Ethics certificate**