EFFECTS OF NATUZYME50® SUPPLEMENTATION ON FIBRE DIGESTION, GROWTH AND CARCASS CHARACTERISTICS OF MALE ROSS 308 BROILER CHICKENS

MASTERS OF AGRICULTURAL MANAGEMENT
(ANIMAL PRODUCTION)

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By

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DECLARATION

I declare that the full-dissertation hereby submitted to the University of Limpopo, for the degree of Masters of Agricultural Management (Animal Production) has not previously been submitted by me for a degree at this or any other university; that it is my work in design and in execution, and that all material contained herein has been duly acknowledged.

______________________ ______________

Surname, Initials (title) **Date**

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ABSTRACT

Two experiments were conducted to determine the effect of natuzyme50® supplementation on fibre digestion, growth and carcass characteristics of Ross 308 broiler chickens aged one to 42 days. The first experiment determined the effect of natuzyme50® supplementation on feed intake, growth and live weight of unsexed Ross 308 broiler chickens aged one to 21 days. Unsexed day old chicks were assigned to 4 different natuzyme50® supplementation levels of 0.0 (UNZ_{0}), 0.75 ($UNZ_{0.75}$), 1.0 ($UNZ_{1.0}$) and 1.5 ($UNZ_{1.5}$) g/kg DM feed, replicated 4 times and each replicate having 10 chicks. A general linear model (SAS, 2008) was used to analyse the data. Ross 308 broiler chickens on a diet supplemented with 1.5g of natuzyme50® per kg DM had higher ($P<0.05$) DM feed intake than those on a diet not supplemented with natuzyme50®. Natuzyme50® supplementation did not ($P>0.05$) have any significant effect on growth rate of unsexed Ross 308 broiler chickens aged one to 21 days. Unsexed broiler chickens on a diet supplemented with 1.5g of natuzyme50® per kg DM had higher ($P<0.05$) live weights than those on a diet not supplemented with natuzyme50® at the age of 21 days

The second experiment determined the effect of natuzyme50® supplementation on feed intake, growth performance and carcass characteristics of male Ross 308 broiler chickens aged 22 to 42 days. The chickens aged 22 days were assigned to 4 natuzyme50® supplementation levels of 0.0 (MNZ_{0}), 0.75 ($MNZ_{0.75}$), 1.0 ($MNZ_{1.0}$) and 1.5 ($MNZ_{1.5}$) g/kg DM feed, replicated 4 times and each replicate having 6 chickens. The diets were isocaloric and isonitrogenous. A general linear model (SAS, 2008) was used to analyse the data obtained. Natuzyme50® supplementation level had no effect ($P>0.05$) on feed intake of male Ross 308 broiler chickens aged 22 to 42 days. However, natuzyme50® supplementation level had effect ($P<0.05$) on growth rate of male Ross broiler chickens aged 22-42 days. Male Ross 308 broiler chickens on a diet supplemented with 1.5g of natuzyme50® per kg DM had higher ($P<0.05$) growth rates than those on a diet supplemented with 1.0g of natuzyme50® per kg DM. However, the significant differences did not extend to birds on a diet not supplemented with natuzyme50®, diets supplemented with 0.75 or 1.5g of natuzyme50® per kg DM which showed similar ($P>0.05$) growth rates. Natuzyme50® supplementation levels used had no effect ($P>0.05$) on carcass, breast and drumstick weights of male Ross 308 broiler chickens. Natuzyme50® supplementation levels had no effect ($P>0.05$) on meat juiciness. However, natuzyme50® supplementation improved ($P<0.05$) meat tenderness and flavour. Increasing natuzyme50® level of supplementation decreased ($P<0.05$) meat shear force values.

It is concluded that natuzyme50® supplementation improved ($P<0.05$) meat tenderness and flavour, and reduced meat shear force values of male Ross 308 broiler chickens. However, further studies are recommended to ascertain the current results.

Keywords: Natuzyme50® supplementation level, Ross 308 broiler chickens, fibre digestion, Performance

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CHAPTER 1

INTRODUCTION

1.1 Background

The poultry industry is South Africa's largest agricultural industry and is a major contributor to the Gross Domestic Products (GDP), employment creation and poverty reduction (Bagopi *et al.,* 2014; Mohammed *et al*., 2018). Poultry production is the quickest way to increase the availability of high quality protein for human consumption (Rafiullah and Sajid, 2013). Mohammed *et al*. (2018) indicated that the growth rate of commercial broiler chickens has improved four-fold and feed efficiency has improved three-fold compared to the situation sixty years ago. However, over the years, the cost of poultry feeds has been on the rise due to several factors, low productivity, insects, weeds, environmental concerns, cost efficiency, sustainability, declining area under cultivation etc (Kshash and Oda, 2019). Falodi *et al.* (2016) indicated that high feed cost development has led farmers to be rationing food for their birds or mixing their feeds with lesser nutritious ingredients. Chuka (2015) and Bokau *et al.* (2018) stated that the high cost of feed ingredients cause farmers to feed poultry with poor quality agro by-products such as palm kernel cake, rice husk and papaya leaves which reduce the efficiency of feed utilization due to high fibre contents.

Research has been done to identify possible feed additives that can assist chickens to utilize these fibrous feed stuffs as chickens do not produce enzymes that are required for the digestion of non-starch polysaccharides (Khattak *et al.,* 2006; Adams and Pough, 1993). The use of exogenous enzymes as feed additives in poultry diets has a history of less than 20 years but has now become a common practice in many countries (Nikam *et al.,* 2017; Rabie and Abo EI-Maaty., 2015; Zaghari *et al.,* 2008). Improved enzymes such as probiotics, prebiotics and phytogenics have been developed over the years and research continues for the search of even more improved enzymes. Natuzyme50® is composed of multi enzymes such as xylanase, cellulase, protease, phytase, alpha-amylase and beta-glucanase (Panda *et al.,* 2011; Larhang and Tork, 2011). Positive effects of natuzyme50® use in poultry feeds as a supplement such as hydroxylation of non-starch polysaccharide (NSP), reducing digesta viscosity, improving nutrient absorption and improving body weight gain have been reported (Mohammed *et al.,* 2018; Gade *et al.,* 2017; Kocher *et al*., 2015; Ahmad *et al.,* 2013; Karimi *et al.,* 2013;

Mushtaq *et al.,* 2009; Zaghari *et al.,* 2008; Shirley and Edwards, 2003). *Bokau (2018), Mareta et al. (2017), Anuradha and Roy (2015), Yoruk et at. (2006), Badawy (1996), Fayek et al. (1995), Vukic and Wenk (1995), Francesch et al. (1994)* and Brenes *et al.* (1993) reported that exogenous enzymes increase feed utilization through increasing digestibility.

The present study focused on the effect of natuzyme50® supplementation on fibre digestion, growth and carcass characteristics of Ross 308 broiler chickens aged one to 42 days.

1.2 Problem statement

Broiler diets are composed mainly of cereals. These cereals contain structural carbohydrates like cellulose and lignin which reduce the digestibility of the diets (Alam *et al.,* 2003). Bokau (2018) defined digestion as the process of breaking down feed into micro-molecules through a series of physical and chemical processes, thus enabling them to be absorbed through the intestinal wall into the capillaries. Broiler chickens are not able to fully digest some of these structural carbohydrates because they do not produce enough enzymes that digest fibre (Nikam *et al.,* 2017; Panda *et al.,* 2011; Buchanan *et al.,* 2007; Khan *et al.*, 2006; Choct *et al.,* 1995). In addition, the lack of enzymes to digest structural carbohydrates in poultry results in lower utilization of other nutrients leading to depressed performance (Ismail *et al,* 2011). Increasing the digestion of these carbohydrates would improve nutrient utilization from such diets (Zou *et al.,* 2013; Novak *et al.,* 2007).

Exogenous enzymes have been used to improve structural carbohydrate digestion by breaking polymeric chains of non-starch polysaccharides and thus improving the nutritive value of the diets (Horvatovic *et al.*, 2015; Hajati, 2010; Morgan and Bedford, 1995). Exogenous enzymes are used in different animal diets such as pigs and ruminants (Suhermiyati and Iriyanti, 2013). Better exogenous enzymes are being developed all the time. One of such enzymes is Natuzyme50® which is composed of multi-enzymes such as xylanase, beta glucanases, cellulases, alpha-amylase, phytase, protease and pectinase (Makinde *et al.,* 2013; Khan *et al.,* 2006). Natuzyme50® (Bioproton PTY, LTD, Sunnybank, Queensland, Australia) is formulated for animals such as cattle, aqua, pigs and poultry. This enzyme does not depend on other enzymes and it can be used as a single dose unlike other enzymes that work in synergy (Panda *et al.*, 2011).

Natuzyme50® cocktail has been tested in fish and pigs with success (Hlophe-Ginindza *et al*., 2016). However, it has not been extensively used in poultry production. Natuzyme50® has beneficial effects of improving the digestibility of cereal-based diets/ feed ingredients by inactivating the anti-nutritional factors and the complex cell wall structure of feeds such as fibre and NSP.

The present study determines the effect of Natuzyme50® supplementation on fibre digestion, growth and carcass characteristics of male Ross 308 broiler chickens aged one to 42 days.

1.3 Scientific contribution of the study

Broiler production plays important economic and nutritional roles in rural households of South Africa. Broiler chicken meat is a source of protein, minerals and vitamins. This study provides information on the effect of Natuzyme50® supplementation on fibre figestion, growth and carcass characteristics of Ross 308 broiler chickens aged one to 42 days. Such information will help farmers, organisations and governments running programs aimed at improving productivity of Ross 308 broiler chickens. Improved productivity of the chickens may result in higher economic and nutritional benefits to farmers raising Ross 308 broiler chickens. Use of Natuzyme50® supplement may results in improved productivity of the broiler chickens (Oliaei *et al.,* 2016; Sharifi *et al.,* 2013).This information will also help in verifying whether Natuzyme50® use in poultry can be effective.

1.4. Aim

The aim of the study was to evaluate the effect of Natuzyme50® supplementation on fibre digestion,   growth and carcass characteristics of Ross 308 broiler chickens aged one to 42days.

1.5 Objectives

The objectives of the study were to determine:

i. the effect of Natuzyme50® supplementation on fibre digestion, feed conversion ratio, live weight and carcass characteristics of Ross 308 broiler chickens aged one to 42 days.

ii. the effect of Natuzyme50® supplementation on gut morphology, and meat quality attributes of Ross 308 broiler chickens aged one to 42 days.

1.6 Hypotheses

The hypotheses of the study were:

i. Natuzyme50® supplementation has no significant effect on fibre digestion, feed conversion ratio, live weight and carcass characteristics of Ross 308 broiler chickens aged one to 42 days.

ii. Natuzyme50® supplementation has no significant effect on gut morphology, and meat quality attributes of Ross 308 broiler chickens aged one to 42 days.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Broiler diets are composed of cereals that contain anti-nutritive factors such as NSP, tannins, glucosinolates and phytic acid that can affect the digestive process (Suhermiyati and Iriyanti, 2013; Khajali and Slominski, 2012; Ward, 1995; NRC, 1994). Nduka (2006) indicated that the majority of these starch present in cereals are not readily digested by monogastric animals and a large proportion of the contents are present as non-starch polysaccharides (NSP). Selvendran and Robertson (1990) reported that almost 90% of the cell walls of plants/ cereals are made up of NSPs such as cellulose, hemicellulose and pectins which are most abundant. Non starch polysaccharides are the main factors that reduce nutrients bioavailability by their different types of fibre such as lignin, arabinoxylan, β-glucans, galactose and mannase in poultry feedstuff (Zou *et al.,* 2013). The viscosity developed by arabinoxylans and β-glucans is the principal factor by which they exert their antinutritive activities. Diets containing cereals are likely to contain anti-nutrients that inhibit or limit utilization by interfering with the digestive process (Ren *et al*., 2012) and these plant materials are a rich source of carbohydrates (Jorgensen *et al.,* 1996). These feed ingredients slow the absorption rate of nutrients in the intestines resulting in high intestinal viscosity. Saki (2005) indicated that high intestinal viscosity reduces the economic value of carcasses, feed intake, weight gain, efficiency of feed utilization and apparent metabolizable energy.

Therefore, exogenous enzymes may be used to inactivate anti-nutritional factors and enhance the nutritional value of plant-based protein in broiler chickens (Dalsgaard *et al.,* 2012). The enzymes required to digest cell wall material, including arabinoxylan, β-glucans and xylanase. They are used as feed additives and are being produced by commercial fermentation processes.

2.2 Fibre digestion in broiler chickens

McDonald and Whitesides (2002) define fibre as a term that refers to cell walls of plant tissue that mostly consist of lignin, cellulose as well as hemicelluloses. Varastegani and Dahlan (2014) indicated two terms that are mostly applied synonymously in animal nutrition as crude fibre and roughage, crude fibre refers to the structural carbohydrates made of cellulose, hemicelluloses and lignin in the plant cell wall while the composition of crude fibre in each single plant is different from other kinds of plants. Dietary fibre inclusion levels range from 3 to 4% but should not exceed 7% for optimal poultry growth performances (Varastegani and Dahlan, 2014). Lower than the recommended level of fibre in chicken diets increases the risk of cannibalism as well as infections of the gastro-intestinal tract. The maintenance of poultry health and production depends on a balanced diet. Some evidence suggests that fibre digestion depends on various factors such as: levels of fibre in the diet, species and age of chickens, ingredient composition and the amount of feed consumed, nutritive value of the diet, level of anti-nutritive factors in the cereal, chicken management and environmental conditions during rearing (Bokau *et al.,* 2018; Mareta *et al.,* 2017; Mateos *et al.,* 2013; Khattak *et al*., 2006).

Dietary fibre plays an important role in the digestive tract of the animal though it does not undergo much digestion in mono-gastric animals due to lack of digestive enzymes that hydrolyse the insoluble NSP (Usama, 2010). Exogenous enzymes are commonly added to the diet or feed by-products to aid fibre digestion or to solubilize phytic phosphorus, thereby reducing their negative effect on animal performance (Alefzadeh *et al*., 2016; Tufarelli *et al*., 2007; Choct *et al*., 1999). McNab and Boorman (2002) reported that NSP exists in two types as soluble (β-glucose, arabinoxylan, arabinogalactose, xyloglucon ect.) and insoluble (cellulose) NSP. Nikam *et al.* (2017) and Ward (1995) indicated that soluble NSPs have the property to immobilize water in their matrix by forming a loose gel network which is responsible for increased viscosity, there by depressing the digestibility of fats, proteins and starch. Insoluble NSP has also a negative impact to the gastro-intestinal tracts of chickens. Gastro-intestinal tract pH levels influence the growth of microbes which affects digestibility and absorption of nutrients (Mabelebele *et al.,* 2014). Furthermore, dietary fibre increases duodenal length which significantly increases nutrient digestion (Oliaei *et al.*, 2016) and improves the levels of healthy gut microflora (Scheideler *et al*, 2005).

2.3 Poultry gut enzymes

Poultry depend on their endogenous enzymes for digestion. The endogenous production of enzymes may be affected by different factors such as age of the chicken, health status, type of feed consumed, environmental factors, type and dose of exogenous enzyme (Bokau, 2018; Faramarzi-Garmroodi, 2014 and Choct *et al.*, 1995). Poultry do not produce enzymes that hydrolyse NSP present in the cell wall of grains (Khattak *et al.,* 2006). They rely on acid digestion in the proventriculus and microbial digestion in the caeca and large intestine (Buchanan *et al.,* 2007). The basal diet of broiler chickens is made up of different feedstuffs such maize, soya, wheat, barley, rice and wheat. In monogastrics, these feedstuffs are not readily digested and hence not available for utilization in metabolic processes due to their high NSP content. Kamran *et al.* (2002) reported that lack of appropriate enzymes in the gastro intestinal tract and presence of anti-nutritional factors hamper the digestion. Buchanan *et al.* (2007) indicated that NSP digestion can range from 13% in lupin diets to 21.9% in wheat diets without enzyme supplementation, while maize and rice brain contains 9 and 25% of NSP, respectively (Nikam *et al*., 2017).

The benefits of enzyme inclusion in poultry diets may resolve the NSP negative effects by improving feed efficiency (Cresswell, 1994). Exogenous enzymes are derived from different sources such as microorganisms and plants (Alagawany *et al.,* 2018[b]). Microorganisms that are generally involved in production of enzymes are: bacteria (*Bacillus subtilis, Bacillus lentus, Bacillus amyloliquifaciens* and *Bacillus stearothermophils*), fungus (*Triochoderma longibrachiatum, Asperigillus oryzae* and *Asperigillus niger*) and yeast (*Saccharomyces cerevisiae*) (Panda *et al.,* 2011; Wallis, 1996). Enzymes that have been used over the past years in the feed industry include: cellulase (ß-glucanases), xylanases and associated enzymes, phytases, proteases, lipases, and galactosidases (Khattak *et al.*, 2006).

2.4 Exogenous enzyme supplementation in poultry diets

The use of exogenous enzymes in poultry production is necessitated by the presence of anti-nutritional materials in poultry feeds (Lee *et al,* 2014). Oliaei *et al.* (2016) reported that many studies have clearly demonstrated that, the use of exogenous enzymes in poultry diets is an effective way to increase the digestibility of complex molecules, especially in young animals, which do not have a well-developed intestinal enzyme profile. Many studies have shown that the negative effect of NSP can be overcome by supplementing a diet with suitable exogenous enzymes (Usama, 2010). Exogenous enzymes hydrolyse the NSP, which leads to digesta viscosity reduction, nutrient absorption improvement and better growth performance (Khattak *et al.*, 2006). The study of Lee *et al.* (2014) supported the statement by indicating the beneficial effect of using the exogenous enzymes in poultry diets to alleviate the anti-nutritional factors and improve the utilization of dietary energy and protein, thus leading to enhanced poultry performance. Ismail *et al* (2011); Bedford and Partridge (2001) reported that the use of exogenous enzymes improve digestion, by removing antinutritional factors which interfere with the normal processes of digestion, by digesting the fibre components of the diet, or by creating an environment which encourages minimal bacterial fermentation in small intestine while encouraging bacterial fermentation in the caeca. The study of Alam *et al.* (2003) reported that the addition of enzymes to the diet reduced feed intake because the chickens meet their nutrient requirements by eating less amount of feed although feed conversion ratio of broiler chickens improved with fed enzyme-based diets. Zanella *et al.* (1999) reported that the excretion of nitrogen (N) in birds fed enzyme based diets decreased and crude protein (CP) digestibility improved by 1.9 %. This is in contrast with the research done by Buchanan (2007) who did not observe any significant effect on performance or digestibility variables when an exogenous enzyme was supplemented in a variety of poultry rations.

2.5 Enzymes used in the poultry industry

Panda *et al.* (2011) defined enzymes as biological catalysts which bring about biochemical reactions without themselves undergoing any change. Enzymes are protein in nature and composed of amino acids arranged in a sequence. They accelerate chemical reactions of the living cells for certain nutrients from feed stuffs to become available to the organs and tissues. Therefore, enzymes are classified by the substrates upon which they react, and by their specificity (Alagawany *et al.,* 2018[b]).

Bedford (2000) indicated three categories of enzymes that are currently being used in the poultry industry as follows: Viscous grain targeted such as Rye, wheat, Oats and barley; Non-viscous grain targeted such as Corn and Sorghum and Phytase. Using enzymes technology is the principle rationale to improve the nutritive value of feedstuffs (Bedford and Partridge 2001). High content of fibre in poultry diets limits the usage of these feed with less nutritional value. Tavernari e*t al.* (2008) suggested enzymes as a solution to solve the problem in poultry diets. Furthermore Ravindran (2013) indicated that the usage of enzymes cause the disruption of the plant cell wall integrity and consequent release of nutrients encapsulated by the cell wall.

2.6 Limitations in the use of enzymes in poultry

Bedford (2000) reported three areas of limitations in the commercial use of enzymes which need further improvement as follows: the efficacy of enzyme preparations and prediction of the enzyme effect, the analysis of enzyme activity in finished feed and thermotolerance of the enzyme preparation.

2.7 Natuzyme50® supplementation in broiler chicken feeds

Natuzyme50® is a high quality and multi-activity feed enzyme formulated specifically for animal feeds to enable better nutrient utilization and resulting in more high quality meat with lower total costs (Panda *et al.,* 2011). Natuzyme50® is a combination of 13 different active enzymes (cellulase, hemicellulase, amyloglycosidase, pentosanase, xylanase, β-Glucanase, α-amylase, protease, pectinase, phytase, lipase, acid phosphatase and acid phytase (Mohammed *et al*., 2018; Lee *et al.*, 2014; Sharifi, 2013; Zamini *et al.,* 2014; Panda *et al.,* 2011 and Lin *et al*., 2007). It is applied to plant-based feeds such as maize, soya, wheat, barley, rice (bran), wheat (bran), and other locally available ingredients as they contain anti-nutritional factors that reduce animal performance.

2.7.1 Benefits of using natuzyme50® in diets.

Natuzyme increases feed intake (khattak *et al.*, 2006). The study done by Bergstrom *et al.* (2007) observed an improved average daily feed intake of pigs in a diet supplemented with natuzyme at a level of 0.035%. These observations are in contrast with the study of Alam *et al.* (2003), Ceylan and Cufadar (2018) who reported reduced feed intakes of birds on diet supplemented with enzymes.

Natuzyme improves nutrient digestibility (Berwanger *et al.*, 2017; Amerah, 2015; Khan *et al.,* 2006). The inclusion of natuzyme, which has NSP enzyme, allows the breakdown of anti-nutritional factors (ANFs) contained in plant-based feeds such as fibres, proteins, starches and phytates to became digestible, these process leads to improved digestion and resulting in greater feed efficiency (Zamini *et al.,* 2014). It is reported that negative effects of NSP oligosaccharides, and phytic acid can be overcome by the supplementation of diets with suitable enzymes (Adeola and Cowieson, 2011). The study done by Adeola and Cowieson (2011) observed an improved nutrient utilization when exogenous enzymes were added to the diets. Alagawany and Attia (2015) reported improved fibre digestion in poultry diets supplemented with commercial enzymes. The results of Panda *et al*. (2011) indicated a positive response following addition of natuzym50® to poultry diets. The application of phytase has been successful in breaking down phytate to increase mineral and nutrient digestibility that in turn improves the growth performance of fish (Adeoye *et al.,* 2016; Kiarie *et al.,* 2013). Classen *et al.* (1995) also reported an improved nutritional value of feed in terrestrial animal when fed exogenous enzyme supplementation.

Natuzyme improves feed conversion efficiency (Panda *et al.,* 2011). The study of Alam *et al.* (2003) reported that the addition of enzymes to the diet reduced feed intake due to birds fulfilling their nutrient requirement by taking less amounts of feed, although feed conversion of broilers improved when fed enzyme based diets, feed cost/kg reduced due to addition of enzymes and meat yield also increased. Similar results were also reported in fish by Hussein *et al.* (2019) who observed an improved FCR in diets containing enzymes compared to the control group.

Natuzyme reduces feed cost (Bioproton, 2007). Nowadays, the feed represents about 75% of the total costs of animal production (Alagawany *et al.,* 2018[b]). Due to high cost of feeds farmers end up utilizing feed ingredients that are not of good quality which result in negative effect than conventional feedstuffs (Alagawany *et al.,* 2018[b]). Natuzyme supplementation in diets allow the use of alternative feed ingredients (raw materials) such as waste of fruits and vegetables after harvesting which are cheaper. Alam *et al.* (2003) reported lower feed cost/kg due to additional of enzymes in diets. Similarly, Bedford (2000) reported that the use of enzyme enables the feed compounder to minimise feed costs through reduced usage of expensive ingredients. In addition Mikulski *et al.* (1999) also reported that enzyme supplementation decreased the relative cost of broiler feeds by 4 to 18% compared to those without enzyme supplement feed.

Natuzyme reduces the incidence of wet litter that commonly happens when diets rich in viscous grains are fed (Yu and Chung 2004). Panda *et al. (*2011) and Usama (2010) reported that since chickens cannot digest diets with high fibre, NSPs end up absorbing large amounts of water and causing an increase in internal viscosity which results in sticky dropping problems. Non starch polysaccharides degrading enzymes such as cellulose, xylanase etc. are capable of disrupting plant cell wall integrity and this enhances rapid digestion by reducing viscosity in the gut (Zijlstra *et al.,* 2007). Similarly, Makinde *et al.* (2013) and Panda *et al.* (2011) reported that the use of enzyme reduces the incidence of wet and sticky droppings, and reduce ingesta viscosity in the intestine. This may reduce the environmental factor such as pollution (Alagawany *et al.*, 2018[b]) due to wet droppings that is caused by poor agro-products (Panda *et al.,* 2011). Poor management of wet droppings may cause serious ill-health in both chickens and human being due to high level of nitrates that develops in poultry waste. Wet droppings also contain bacteria that contaminate drinking water.

Natuzyme reduces phosphorus and nitrogen excretion (Rehman *et al.,* 2017; Abd El-Hack *et al.,* 2017[a]; Malakzadegan *et al.,* 2012). Most of the phosphorus contained in animal feed of plant origin exists in the storage form phytate. Monogastric animals cannot digest phosphorus contained within phytate, since they lack phytase enzyme that breaks down this phytate molecule. Therefore the inclusion of the phytase enzyme in natuzyme is required for release of phytate-bound phosphorus (Adeoye *et al.,* 2016). This enable the reduction of inorganic phosphorus, which is most commonly applied in the form of dicalcium phosphate (DCP) or bone meal into animal feed. The study of Zanel *et al.* (1999) revealed that the excretion of nitrogen (N) in birds fed enzyme based diets decreased and crude protein (CP) digestibility improved by 1.9 times. Salami *et al.* (2015), reported highest phosphorus and fat retention respectively, when broilers were fed a test diet supplemented with Roxazyme G2® and natuzyme®. Similarly, Panda *et al.* (2011) and Rehman *et al.* (2017) showed that an increased use of enzymes enhances nutrient utilization by reducing the manure output and reducing nutrient excretion particularly excess phosphorus, nitrogen, copper and zinc.

Natuzyme improves animal wellbeing, reduces mortality and need for antibiotic use (Panda *et al.,* 2011). Adeyemo and Onikayi (2012) indicated that as broiler diets are composed of raw materials that are not properly processed, they may lead to health problems and reduction of production. Thirumalaisamy *et al.* (2016) and Zakaria *et al.* (2010) reported that inclusion of supplements in diets is essential as they provide essential nutrients necessary for health and performance.

Natuzyme improves live weight gain (Panda *et al*., 2011 and Attia *et al.,* 1998). Supplementation of Natuzyme in diets with low, moderate and high fibre were investigated by various researchers. Zamani *et al.* (2017), Oliaei (2016), Zeng *et al.,* (2015), Milanloo *et al.* (2012), Chimote *et al.,* (2009) found a significant improvement in live weight of broiler chickens feed on diets containing Natuzyme. Raza *et al.* (2009) reported improved live weight of broiler chickens fed enzyme supplemented diets containing 6% crude fibre compared to diets without enzyme. Zaghari *et al*. (2008) reported that Natuzyme50® inclusion in diets has a positive effect on broiler performance. Similar results were also reported by Zamini *et al.* (2014), Kocher *et al*. (2003) who observed positive effect of various commercial multi-enzyme complex (phytase,xylanase, β-glucanase, amylase, cellulose and pectinase) on the growth performance of *Lateolabrax japonicas.* In addition, the results observed by Hussein *et al.* (2019) showed that dietary inclusion of 2% of Natuzyme improved growth performance. The above findings are in contrast with the observations reported by various researchers who did not observe any improved growth performance in poultry when diets supplemented with enzymes were fed to poultry (Ahmed *et al.,* 2017; Rabie and Abo EI-Maaty, 2015; Slominski 2011 and Shalash *et al.,* 2009). Similarly, Sharifi *et al.* (2013) and Mikhail *et al.* (2013) showed that growth performance was not improved by adding exogenous enzyme. Yigit *et al.* (2014) also did not observe any improvement on the growth performance when a fish feed mixed with exogenous enzyme was fed to rainbow trout (*Oncorhynchus mykiss* (Walbaum).

Natuzyme improves meat quality (Loddi *et al.,* 2000). The major poultry meat quality attributes are appearance, texture, juiciness, flavour and functionality (Petracci and Baeza, 2009). Major components of meat quality are indicated in Table 2.01. Chinese consumers determine meat quality of chicken by the flavour, juiciness and colour (Jiang and Groen, 2000). Flecher (2002) reported two most important quality attributes for poultry meat as appearance and texture.

Texture is the attribute that is measured by tenderness or toughness of the meat (Petracci and Baeza, 2009). It is evaluated on meat cuts after cooking under standardized conditions. Lawrie (1998) defined tenderness as the ease of mastication, which involves the initial ease of penetration by teeth, the ease with which the meat breaks into fragments and the amount of residues remaining after mastication. Texture is the most important sensory property affecting final quality assessment (Wood *et al.,* 1996). Meat tenderness was primarily associated with live bird quality factors such as breed, sex, feeding, age, slaughter technology, cooling and storage (Ristic *et al.,* 2010).

Table 2.01 Major components of meat quality

Components	**Meat quality factors**
Yield and gross composition	Muscle quality and size
	Ratio of fat to lean meat
Appearance and technological characteristics	Chemical composition and marbling of lean meat
	Fat texture and colour
	Colour and water holding capacity of lean meat
Palatability	Texture and tenderness
	juiciness
	Flavour
Wholesomeness	Chemical and microbial safety
	Nutritional quality
Ethical quality	Acceptable animal husbandry

(Warris *et al*., 2000)

Juiciness of the meat is mainly related to the water holding capacity of the meat or low marbling fat level. People prefer chewable meat juiciness and low marbling fat level complemented with flavour and adour. Flavour and pleasant adour determines the quality of meat, whether it is fresh or spoiled with unpleasant smell (Warris, 2000).

Meat flavour is another attribute that consumers use to determine the acceptability of chicken meat. Taste and odour contribute to the flavour of poultry meat and it is difficult to differentiate between the two during consumption. Meat flavour is affected during production and processing. Meat flavour is related to bird strain, environmental conditions, diet, chilling, scalding temperatures, product packaging and storage (Flecher, 2002).

2.8 Alternatives for exogenous enzyme supplementation in poultry feeds

An effective alternative use of exogenous enzyme supplementation in poultry should comply with certain characteristics. Alternatives have to be approved and used in poultry feeds without threat to humans and the environment. Bedford and Schulze (1998) stated that there are undoubtedly far better, more efficient and much cheaper alternative enzymes on the market and emphasis was made as alternatives and have to satisfy the demand for broiler production.

Enzyme alternatives should be made from natural products of fermentation and be able to produce significant beneficial impact on animal production output. The study of Lee *et al.* (2014) indicated the most widely accepted alternatives as follows: probiotics, prebiotics and phytogenics including plant extracts.

2.9 Factors affecting responses to dietary exogenous enzyme supplementation in poultry

Positive responses of animal production output should be shown in digestion, absorption, animal health and overall improved performance (Khattak *et al.,* 2006). The studies by Bokau (2018), Khattak *et al*. (2006) and Cowieson *et al.* (2006) showed that the responses to exogenous enzyme supplementation depend on the type of animal (poultry tend to be more responsive to dietary enzyme supplementation compared to pigs), age of the animal (young animals tend to respond better to enzymes than older animals), type of gut micro flora present in the gastrointestinal tract and the physiological status of the bird, quality or type of diet used and concentrations of enzymes used. The study done by Zamini *et al.* (2014) revealed that response depends on concentration (relative to body weight) and the direction (positive or negative of an animal's response to supplementation).

Natuzyme has many enzyme activities included in the feed mix (Panda *et al.,* 2011). Table 2.02 summarizes the various responses obtained from addition of different feed enzymes to poultry diets (Swiatkiewicz *et al.* (2015).

2.10 Mode of action of exogenous enzymes

Different modes of action for exogenous enzymes have been reported by various researchers (Dida, 2016; Swiatkiewicz *et al.,* 2015; Ravindran 2013; De Vries *et al.* (2012; Slominski, 2011; Adeola and Cowieson, 2011; Khattak *et al.,* 2006). Bedford and Schulze (1998) suggested that since broiler diets contain mainly cereals that have high fibre/ starch in the form of NSP, exogenous enzymes may work by reducing the viscosity of the digesta in the gastro intestinal tract. These cereals contain soluble indigestible polysaccharides like arabinoxylan which is anti-nutritional, this limits their feed value and use. Salami (2015) and Panda *et al.* (2011) reported these arabinoxylans to be present in cereals like wheat, rye and triticales. However non-starch polysaccharides have a detrimental effect on gastrointestinal tract function because they; bind lots of water, increase viscosity in the gut, reduce passage rate of digesta, growth rate, feed intake and nutrient availability (Kalantar *et al.*, 2015; khattak *et al.,* 2006).

Exogenous enzyme supplementation in poultry diets improves bird performance by increasing effectiveness of host (endogenous) enzymes, breaking down the gel form characteristic of soluble fibres which allows the birds' digestive enzymes to function more efficiently. This improves starch, protein, fat, amino acids and energy digestibility (Bokau *et al*., 2018), altering the feed passage rate thereby reducing the water holding capacity of the gut contents thus increasing the dry matter content and stimulating feed intake, increasing phosphorus (P) digestibility by 20-50% and resulting in marked decrease in phosphorus excretion (Tang *et al.*, 2014; Woyengo and Nyachoti, 2011).

Table 2.02 Summary of responses to addition of different feed enzymes to poultry diets

Production stage	**Enzyme**	**Enzyme Responses**	**Reference**
Broilers, 8–21 days of age	Phytase	Improved phosphorus bioavailability but no consistent effects on ME content and amino acid digestibility	Martinez-Amezcua *et al.,* 2006
Broilers, 1–21 days of age	Carbohydrases, protease, phytase	Phytase improved dry matter and N digestibility but enzymes had no effect on growth performance	Olukosi *et al.,* 2010
Broilers, 1–42 days of age	Xylanase	Improved feed intake, body weight gain, and dry matter, protein, and hemicellulose digestibility	Liu *et al.,* 2011
Broilers, 18–23 days of age	Multi-enzyme containing xylanase, β-glucanase, mannanase, and phytase	No effect on growth performance or nutrient digestibility	Min *et al.,* 2011
Broilers, 12–21 or 7–21 days of age	Multi-enzyme 1 (xylanase, amylase) Multi-enzyme 2 (xylanase, amylase, protease)	Both multi-enzymes improved ME, and Multi-enzyme 2 improved amino acid digestibility except methionine	Romero *et al.,* 2013
Broilers, 1–42 days of age	Xylanase, phytase	Combination of enzymes improved growth performance, dry matter and organic matter digestibility, retention of Ca and P, and bone characteristics	Swiatkiewicz *et al.,* 2014b

Source: Swiatkiewicz *et al.* (2015)

Bedford and Schulze (1998) stated that dietary enzymes hydrolyse NSP and make nutrients available for animals by breaking the cell structure and release starch that increase metabolisable energy that reduce intestinal viscosity. Protein release by action of proteases may lead to maximum absorption of minerals and may result in improvement in nutrient utilization. Zaghari *et al.* (2008) suggested that the use of enzymes such as proteases in addition to cell wall-degrading enzymes may be even more effective. Bedford and Schulze (1998) stated that dietary supplementation of exogenous phytase makes phosphorus available and improves it's digestibility by breaking the cell wall matrix, and making provision of substrates for beneficial microflora.

2.11 Conclusion

Chicken meat is used world-wide as a convenient and affordable source of protein for human beings. Enzymes are used in feeds to increase productivity of chickens. Researchers have shown that exogenous enzyme supplementation as an alternative to endogenous enzymes can improve growth performance, decrease production of wastes and improve animal welfare. Other studies have not clearly indicated the impact of enzymes on the productivity and health of chickens. There is a need to conduct more research on the effect of natuzyme50® supplementation to evaluate whether such an enzyme can be used to enhance the digestibility of fibre based-diets so as to complement the endogenous enzymes of broiler chickens. Information on natuzyme50® use in Ross 308 broiler chickens is unclear and inconclusive so, further research is required. Thus, the aim of this study was to evaluate the effect of natuzyme50® supplementation on intake, digestion, growth and carcass characteristics of Ross 308 broiler chickens aged one to 42 days.

CHAPTER 3

MATERIALS AND METHODS

3.1 Study site

This study was conducted at the University of Limpopo Animal Unit, Limpopo Province, South Africa**.** The coordinates of the area are 23°54'00"S and 29°27'00"E. The ambient temperatures at the study site range between 20 and 36°C during summer months and between 10 and 25°C during winter months. The mean annual rainfall is between 445.8 and 456.4 (Shiringani, 2007).

3.2 Preparation of the poultry house

Prior to the chicks' arrival, the experimental house was thoroughly cleaned and disinfected with Vet GL 20 (NTK Company, Polokwane). The house was left to dry for two weeks after cleaning to eradicate the population of infectious microorganisms that were not killed by the disinfectant. Feeders and drinkers were thoroughly cleaned and disinfected before use with the same disinfectant used above. The house was divided into 16 floor pens of $2m^2$ each. Each pen was well-equipped with both feeders and waterers. Wood shavings which were dispersed and spread on the floor to a depth of 7cm were used as bedding. The house was warmed to 36°C with electric brooders and humidity was set at 80% before the arrival of the birds (Amao, 2018). The heating system was provided using 250 watt-infrared lights. The chicks were reared in an open sided house with curtains for fresh air ventilation, reduction of moisture and other gases formed within the house. Feed and clean water were distributed within the rearing pens and properly adjusted. The day-old chicks were received and handled gently to the rearing pens where the initial live weights were measured using an electronic weighing scale.

3.3 Acquisition of materials and chickens

Two hundred day-old Ross 308 broiler chicks were purchased from Lufafa hatchery, Tzaneen, Limpopo Province, South Africa. The chicks were vaccinated at the hatchery with the Vitabron vaccine strain for prevention of Newcastle and infectious bronchitris diseases. The required ingredients to formulate starter and grower diets were acquired from Voorslagvoere Milling Company (Pty) in Mokopane, South Africa. Natuzyme50®, a multi enzyme complex, was acquired from-Bioprotin, Australia. It is composed of alpha-Amylase 3 457 cu/g, beta-Glucanase 6 462 xu/g, phytase 12 975 bu/g, cellulase 48 750 U/g, xylanase 82 750 U/g, protease 5 376 U/g. All house hold disinfectants, medicines, vaccines, feeders, drinkers and 250 watts infrared lights were acquired from NTK (Pty) in Polokwane, South Africa before the commencement of the study. Vaccines were acquired from the Limpopo Department of Agriculture (Veterinary Services).

3.4 Experimental procedures, dietary treatments and design

3.4.1 Dietary treatments for unsexed Ross 308 broiler chickens aged one to 21 days

The first part of the study was to determine the effect of natuzyme50® supplementation on feed intake, growth rate, feed conversion ratio (FCR), live weight, metabolisable energy (ME) intake and nitrogen retention of Ross 308 broiler chickens aged one to 21 days. A total number of 160 day-old-unsexed chicks were assigned to treatments in a randomized design having 4 treatments (0, 0.75, 1.0 or 1.5g of natuzyme50®/kg DM feed), replicated four times and having ten chickens per replicate (Table 3.01).

The experimental chicks were fed starter diets formulated from the feed ingredients with different natuzyme50® levels as indicated in Table 3.02. All the diets contained 21% crude protein, 16MJ of energy/kg DM and 4% CF. The diets met the poultry nutritional standards of the NRC (1994).

The chickens were supplied with fresh feed and water *ad-libitum* throughout the study. Light was provided 24 hours daily. The chickens were monitored daily for signs of ill-health and sick birds were removed from the study. The chicks were subjected to similar managerial practices until they were 21 days of age. The natuzyme50® supplementations included high and low levels as indicated in the literature (Hlophe-Ginindza *et al*., 2016).

3.4.2 Dietary treatments for male Ross 308 broiler chickens aged 22 to 42 days

The second part of the study was to determine the effects of natuzyme50® supplementation on feed intake, growth rate, feed conversion ratio (FCR), live weight, metabolisable energy (ME) intake, nitrogen retention (N-retention) and carcass characteristics of male Ross 308 broiler chickens aged 22 to 42 days. Prior to the start of this experiment the chickens had been fed a diet containing 22g CP/kg DM and 16 MJ of energy per kg DM, meeting their nutritional requirements according to NRC (1994).

Table 3.01 Dietary treatments for Experiment 1 (unsexed Ross 308 broiler chickens aged one to 21 days)

Diet Code	Diet description
UNZ $_{0}$	Diet containing 21% CP, 16 MJ energy/kg DM and 4% CF without Natuzyme50® fed to unsexed broiler chickens aged one to 21 days.
UNZ $_{0.75}$	Diet containing 21% CP, 16 MJ of energy/kg DM and 4% CF and supplemented with 0.75g of Natuzyme50®/kg DM fed to unsexed broiler chickens aged one to 21 days.
UNZ $_{1.0}$	Diet containing 21%CP, 16 MJ of energy/kg DM and 4% CF and supplemented with 1.0g of natuzyme50®/kg DM fed to unsexed broiler chickens aged one to 21 days.
UNZ $_{1.5}$	Diet containing 21% CP, 16 MJ of energy/kg DM and 4% CF and supplemented with 1.5g of Natuzyme50®/kg DM fed to unsexed broiler chickens aged one to 21 days.

CP: Crude protein, MJ: Mega joules, DM: Dry Matter and CF: Crude Fibre

A total number of 96 male chickens, weighing an average of 630 ± 12.0g each were used in a completely randomized design (SAS, 2008) having 4 treatments, replicated 4 times and having six chickens per replicate. The four diets contained 0, 0.75, 1.0 or 1.5g of natuzyme50® per kg DM (Table 3.03).

The experimental chickens were fed diets formulated from the feed ingredients with different natuzyme50® levels as indicated in Table 3.04. All the diets contained 20% crude protein, 16.92 MJ of energy /kg DM and 5% CF. The diets met the poultry nutritional standards of the NRC (1994). The chickens were supplied with fresh feed and water *ad-libitum* throughout the study.

Table 3.02: Ingredients and nutrient composition of diets for Experiment 1

Feed Ingredients	Treatment*			
	UNZ_0	$UNZ_{0.75}$	$UNZ_{1.0}$	$UNZ_{1.5}$
Yellow maize (%)	39.83	39.83	39.83	39.83
Soybean (%)	17.73	17.73	17.73	17.73
Wheat bran (%)	15.00	15.00	15.00	15.00
Sunflower (%)	12.39	12.39	12.39	12.39
Fishmeal (%)	5.66	5.66	5.66	5.66
Vitamin+minerals Premix (%)	3.00	3.00	3.00	3.00
Oil- sunflower (%)	2.50	2.50	2.50	2.50
Sodium bicarbonate (%)	1.50	1.50	1.50	1.50
Limestone (%)	1.50	1.50	1.50	1.50
Salt (%)	0.30	0.30	0.30	0.30
Monocalcium phosphate (%)	0.20	0.20	0.20	0.20
DL methionine (%)	0.15	0.15	0.15	0.15
L threonine (%)	0.15	0.15	0.15	0.15
L lysine (%)	0.10	0.10	0.10	0.10
Terramycin (%)	0	0.01	0	0
Total	100	100	100	100
Nutrient Composition				
Crude Protein (%)	21	21	21	21
Energy (MJ/kg DM)	16	16	16	16
Crude Fibre (%)	4	4	4	4
Fat (%)	6.89	6.01	6.41	3.43
Ash (%)	7.73	8.27	8.42	6.87
Ca (%)	1.02	1.02	1.01	1.01

* : Dietary treatments contained 0, 0.75, 1.0 and 1.5g of natuzyme50® per kg DM feed

Light was provided 24 hours daily. The chickens were monitored daily for signs of ill-health and sick birds were removed from the study. The chickens were subjected to similar managerial practices until they were 42 days of age.

Table 3.03 Dietary treatments for Experiment 2 (male Ross 308 broiler chickens aged 22 to 42 days)

Diet Code	Diet description
MNZ_0	Diet containing 20% CP, 16.92 MJ of energy/kg DM and 5% CF without Natuzyme50® fed to male broiler chickens aged 22 to 42 days.
$MNZ_{0.75}$	Diet containing 20% CP, 16.92 MJ of energy/kg DM and 5% CF and supplemented with 0.75g of Natuzyme50®/kg DM fed to male broiler chickens aged 22 to 42 days.
$MNZ_{1.0}$	Diet containing 20% CP, 16.92 MJ of energy/kg DM and 5% CF and supplemented with 1.0g of natuzyme50®/kg DM fed to male broiler chickens aged 22 to 42 days.
$MNZ_{1.5}$	Diet containing 20% CP, 16.92 MJ of energy/kg DM and 5% CF and supplemented with 1.5g of Natuzyme50®/kg DM fed to male broiler chickens aged 22 to 42 days.

CP :Crude protein, MJ: Mega joules, DM: Dry matter and CF: Crude Fibre

:MNZ_0: 0 g of Natuzyme50®/kg DM feed; $MNZ_{0.75}$: 0.75g of Natuzyme50®/kg DM feed; $MNZ_{1.0}$: 1.0g of Natuzyme50®/kg DM feed; $MNZ_{1.5}$: 1.50g of Natuzyme50®/kg DM feed.

3.5 Data collection

All the measurements done were applied in both Experiments 1 and 2.

3.5.1 Live weight measurements

Mean live weights were calculated from the weekly measurements by dividing the total weight with the number of chickens in the pen. Weekly average body weight gain was obtained by calculating the difference between previous week average body weight and the present week average body weight (Amao, 2018).

3.5.2 Growth rate measurements

Average daily gains were calculated by subtracting the initial weight of the chicken from the final weight = (Final weight – Initial weight)/number of days.

3.5.3 Feed intake measurements

The voluntary feed intake was measured by subtracting the weight of leftovers from that offered per day and the total divided by the number of chickens per pen. An electronic scale was used to measure both feed offered per day and leftovers.

3.5.4 Feed conversion ratio measurements

Daily average feed intake and weight gain were used to calculate feed conversion ratio (FCR). Average feed intake was divided by average gain to get the FCR value (McDonald *et al.*, 2010). Bokau *et al.* (2018) defined feed conversion ratio as the index of the total feed input for growth or the amount of grams required to produce one gram of the weight.

3.5.5 Digestibility measurements

A digestibility trial was carried out at the ages of 35-42 days using specially designed metabolic cages. The cages were equipped with separate feed and water troughs. One chicken was randomly selected from each replicate and transferred to the metabolic cage for the measurement of apparent digestibility. A three-day acclimatization period was allowed prior to a four-day total faecal collection period (Khan *et al.,* 2006). Droppings voided by each chicken were collected daily at 9:00 hours. Care was taken to avoid faecal contamination from feathers, scales and feeds. Apparent digestibility (AD) of the nutrients was calculated according to the procedures of McDonald *et al.* (2010) using the formula below:

AD (%) = Amount of nutrient ingested – amount of nutrient excreted) / amount of nutrient ingested x 100.

3.5.6 Measurements of carcass characteristics, gastrointestinal lengths and weights

Following blood sample collection, the chickens were euthanized according to the guidelines for humane slaughter of chickens provided by the University of Limpopo Committee on Animal Ethics (AREC). Carcass characteristics (carcass and organ weights, organ pH and gastro-intestinal lengths and weights) were determined. Before slaughter each chicken was weighted using an electronic weighing balance. The carcasses were then put inside a bucket containing hot water at 130F (54.4°C) for a 40 seconds and they were then taken out. The carcasses were then put on a table and were hand defeathered

The carcasses were cut open at the top of abdominal site and the digestive tracts were removed from the abdominal cavity. Measurements were taken on weight and length of the whole gut segment. The gut length were measured from the crop up to the caecum. The weight and length of the small intestines, large intestines and caecum were measured separately. Some of the following organs: crop, gizzard, proventriculus and liver were cleaned and then their weights and lengths were measured accordingly, using an electronic weighing scale and measuring tape. Carcass weight, breast, drumstick and thigh weights were weighed and recorded. The effect of natuzyme50® on meat shear force value was determined using a texture analyser equipped with a Warmer-Bratzler shear force apparatus (American Meat Science Association, 1995). Thereafter, meat samples were taken and stored in a refrigerator for dry matter analysis and sensory evaluation.

3.5.7 pH measurements

Crison (Basic 20 pH meter) was used to measure organ digesta pH of the crop, gizzard, proventiculus, small intestines, large intestines and caecum.

Table 3.04: Ingredients and nutrient composition of grower diets for Experiment 2

Feed Ingredients	Treatment*			
	MNZ_0	$M\ NZ_{0.75}$	$MNZ_{1.0}$	$MNZ_{1.5}$
Yellow maize (%)	39.83	39.83	39.83	39.83
Soybean (%)	17.73	17.73	17.73	17.73
Wheat bran (%)	15.00	15.00	15.00	15.00
Sunflower (%)	12.39	12.39	12.39	12.39
Fishmeal (%)	5.66	5.66	5.66	5.66
Vitamin+minerals Premix (%)	3.00	3.00	3.00	3.00
Oil- sunflower (%)	2.50	2.50	2.50	2.50
Sodium bicarbonate (%)	1.50	1.50	1.50	1.50
Limestone (%)	1.50	1.50	1.50	1.50
Salt (%)	0.30	0.30	0.30	0.30
Monocalcium phosphate (%)	0.20	0.20	0.20	0.20
DL methionine (%)	0.15	0.15	0.15	0.15
L threonine (%)	0.15	0.15	0.15	0.15
L lysine (%)	0.10	0.10	0.10	0.10
Terramycin (%)	0	0.01	0	0
Total	100	100	100	100
Nutrient Composition				
Crude Protein (%)	20	20	20	20
Energy (MJ/kg DM)	16.92	16.92	16.92	16.92
Crude Fibre	5	5	5	5
Fat (%)	4.51	4.95	5.37	5.23
Ash (%)	6.45	6.77	6.66	6.32
Ca (%)	1.01	1.02	1.01	1.01

* : Dietary treatments contained 0, 0.75, 1.0 and 1.5g of natuzyme50® per kg DM feed

3.6 Sensory evaluation

Meat samples were frozen at -40°C for 4 days and thawed for 7 hours at room temperature prior to cooking for sensory evaluation. Pavelkova *et al.* (2013) method was applied to prepare the meat. An oven was set at 180°C and allowed to preheat prior to cooking. Thighs and drumsticks were prepared, put in trays and covered with an aluminium foil to prevent water loss. The meat samples were free from any additives.

Prepared meat samples were put in an oven for approximately 60 minutes and turned every 10 minutes. A tong was used for turning to avoid piercing that could cause the moisture to evaporate. Samples were cut into small 5cm cubic pieces and served immediately after cooking for sensory evaluation by twenty selected and trained University of Limpopo postgraduate students according to Pavelkovà *et al.* (2013) and described in Table 3.05. Lemon juice and water were used to rinse and cleanse the mouth before moving to the next sample to avoid confusion of tastes (Príbela, 2001). The following sensory attributes were evaluated by the sensory panel of trained postgraduate students: tenderness, juiciness and flavour of meat samples.

Table 3.05 Sensory evaluation score used for meat testing

	Sensory attributes		
Score	Tenderness	Juiciness	Flavour
1	Too tough	Too dry	Very bad flavour
2	Tough	Dry	Poor flavour
3	Neither tough nor tender	Neither dry nor juicy	Neither bad nor good flavour
4	Tender	Juicy	Good flavour
5	Too tender	Too Juicy	Very good flavour

Source: Palvelkovà *et al*. (2013)

3.7 Shear force

Shear force assessment was done according to Warner-Bratzler Shear Force determination procedures (Dawson *et al.,* 1991). Frozen samples of chicken breast meat were thawed for 24h at 2°C. Thawed samples were removed, tagged and used for cooked Warner Bratzler Shear Force (WBSF) measurements. An electric stove was set at 180°C prior to preparation for 25 min. Breast meat cuts were cooked with an electric stove using boiling method in a cylindrical pot. The cuts were boiled to an internal temperature of 35°C, then turned and finished at 70°C. Cooked cuts were cooled down to room temperature of 18°C for 2 hours before WBSF measurements. Three cylindrical samples (12.5 mm core diameter) each cut were cored parallel to the grain of the meat and sheared perpendicular to the fibre direction using a Warner-Bratzler share device mounted on a Universal Instron Apparatus (cross head speed= 200mm / min, one shear in the centre of each core). The reported value in kg represents the average of three peak force measurements of each sample.

3.8 Chemical analysis

Dry matter contents of the feeds, feed refusals excreta and meat samples were determined by drying the sample at the temperature of 105°C for 24 hours. Ash content of the feeds, faeces, feed refusals and meat samples were analysed by ashing the sample at 600°C in a muffle furnace overnight. Nitrogen contents of the sample was determined by a micro-Kjedahl method (AOAC, 2008). Amino acid and fatty acid contents of feeds and meat were analysed by ion-exchange chromatography at the University of Limpopo (Özlem, 2013). Gross energy values for feeds, feed refusal and faeces were determined using an adiabatic bomb calorimeter at the University of Limpopo Animal Nutrition Laboratory, according to the method described by the Association of Analytical Chemists (AOAC, 2010). A full analysis for faeces and feeds was performed at the Pietermaritzburg laboratory, Kwa-Zulu Natal, South Africa, according to AOAC (2012)

3.9 Statistical analysis

Data on feed intake, live weight, growth rate, digestibility, feed conversion ratio, metabolisable energy, gastrointestinal morphology, shear force, sensory evaluation values and carcass characteristics of male Ross 308 broiler chickens were analysed using General Linear Model (GLM) procedures of the statistical analysis of variance, Version 9.3.1 software program (SAS, 2008) to detect dietary treatment effects. Where significant differences were observed, the mean separation was done using the Tukey test at the 5% level of significance (SAS, 2008). The model $Y_{ij} = \mu + T_i + e_{ij}$, was applied, where Y_{ij} = j^{th} is the observation of the i^{th} treatment level; μ = the overall mean; T_i = the effect of the i^{th} treatment level and e_{ij} = random error.

Responses in optimal dry matter, digestibility, live weight, growth rate, feed conversion ratio, gut morphology, tenderness and juiciness to natuzyme50® supplementation levels were modelled using the following quadratic equation (SAS, 2008):

$Y = a+b_1x + b_2x^2 + e$

Where Y = optimal dry matter, live weight, digestive tract organ size, digestive organ digesta pH values, crude protein and fat digestibility, crop weight, breast meat weight, meat tenderness, juiciness and flavour; a = intercept; b = coefficients of the quadratic equation; x = natuzyme50® supplementation levels; and $-b_1/2b_2$ = x value for optimal response and e is the error.

The relationship between natuzyme50® supplementation level and live weight, meat tenderness, flavour and shear force were modelled using a linear regression equation in the form of:

$Y = a + bx$

Where Y = live weight, meat tenderness, flavour and shear force; a = intercept; b = coefficient of the linear equation; and x = Natuzyme50® supplementation level.

CHAPTER 4

RESULTS

4.1 Unsexed Ross 308 broiler chickens aged one to 21 days (Experiment 1)

Results of the nutrient composition of the diets are presented in Table 4.01. The diets had similar protein level of 21% and energy content of 16 MJ/kg DM as recommended by NRC (1994) for broiler chickens aged 1 to 21 days. The diets had different natuzyme50® supplementation levels of 0.0, 0.75, 1.0 and 1.5g/kg DM feed.

Table 4.01 Diet composition (% except MJ/kg DM for energy) for Experiment 1

Feed Nutrient	Treatment#			
	UNZ_0	$UNZ_{0.75}$	$UNZ_{1.0}$	$UNZ_{1.5}$
DM	90	90	90	90
CP	21	21	21	21
Energy	16	16	16	16
ADF	5.71	5.71	4.92	4.72
NDF	19.07	16.16	13.89	25.48
Fat	6.89	6.01	6.41	3.43
Ash	7.73	8.27	8.42	6.87
Ca	1.02	1.02	1.01	1.01

DM: Dry matter, CP: Crude protein, ADF: Acid detergent fibre, NDF: Neutral detergent fibre and Ca: Calcium

:UNZ_0: 0 g of Natuzyme50®/kg DM feed; $UNZ_{0.75}$: 0.75g of Natuzyme50®/kg DM feed; $UNZ_{1.0}$: 1.0g of Natuzyme50®/kg DM feed; $UNZ_{1.5}$: 1.50g of Natuzyme50®/kg DM feed.

Results of the effect of natuzyme50® supplementation level on feed intake, growth rate, live weight, feed conversion ratio (FCR) and metabolisable energy (ME) intake of unsexed Ross 308 broiler chickens aged one to 21 days are presented in Table 4.02. Ross 308 broiler chickens on a diet supplemented with 1.5g of natuzyme50® per kg DM had higher ($P<0.05$) DM feed intake than those on a diet not supplemented with natuzyme50®. However, chickens on diets supplemented with 0.75, 1.0 or 1.5g of natuzyme50® per kg DM had similar ($P>0.05$) DM feed intakes. Similarly, chickens on a diet not supplemented with natuzyme50® and those on diets supplemented with 0.75 or 1.0g of natuzyme50® per kg DM had the similar (P>0.05) DM intake.

Natuzyme50® supplementation did not (P>0.05) have any effect on growth rate, FCR and N-retention of unsexed Ross 308 broiler chickens aged one to 21 days. Unsexed broiler chickens on a diet supplemented with 1.5g of natuzyme50® per kg DM had higher (P<0.05) live weights than those on a diet not supplemented with natuzyme50®. However, chickens on diets supplemented with 0.75, 1.0 or 1.5g of natuzyme50® per kg DM had similar (P>0.05) live weights. Similarly, chickens on a diet not supplemented with natuzyme50® or on diets supplemented with 0.75 or 1.0g natuzyme50® of per kg DM had the same (P>0.05) live weights.

Metabolisable energy intakes of unsexed Ross 308 broiler chickens aged one to 21 days were affected (P<0.05) by natuzyme50® supplementation level (Table 4.02). Unsexed Ross 308 broiler chickens on a diet not supplemented with natuzyme50® or on diets supplemented with 1.0 or 1.5g of natuzyme50® per kg DM had higher (P<0.05) ME intakes than those on a diet supplemented with 0.75g of natuzyme50® per kg DM. However, chickens on a diet not supplemented with natuzyme50® or on diets supplemented with 1.0 or 1.5g of natuzyme50® per kg DM had similar (P>0.05) ME intakes.

Results of the effects of natuzyme50® supplementation level on optimal dry matter and ME intakes of unsexed Ross 308 broiler chickens aged one to 21 days are presented in Table 4.03 . Feed and ME intakes of unsexed Ross 308 broiler chickens were optimized at supplementation levels of 2.094 (r^2 =1.000) and 1.464 (r^2 =0.980) g of natuzyme50® per kg DM feed, respectively (Figures 4.01 and 4.02, respectively). A positive relationship (r = 0.875) was observed between natuzyme50® supplementation level and live weight of Ross 308 broiler chickens aged 21 days (Figure 4.03).

Table 4.02 Effect of natuzyme50® supplementation level on DM feed intake (g/bird/day), growth rate (g/bird/day), feed conversion ratio, live weight (g/bird aged 21), ME intake (MJ/kg DM) and nitrogen retention (g/bird/day) of unsexed Ross 308 broiler chickens aged one to 21 days*

Variable	Treatment #			
	UNZ_0	$UNZ_{0.75}$	$UNZ_{1.0}$	$UNZ_{1.5}$
DM intake	$83^b \pm 4.3$	$92^{ab} \pm 2.0$	$94^{ab} \pm 3.6$	$97^a \pm 1.0$
Growth rate	$28^a \pm 1.6$	$30^a \pm 0.7$	$29^a \pm 3.4$	$30^a \pm 1.9$
FCR	$28^a \pm 0.15$	$25^a \pm 0.02$	$28^a \pm 0.09$	$25^a \pm 0.10$
Live weight	$430^b \pm 23.7$	$470^{ab} \pm 5.1$	$452^{ab} \pm 28.2$	$530^a \pm 26.9$
ME intake	$10.3^a \pm 0.03$	$11.0^b \pm 0.06$	$11.3^a \pm 0.02$	$11.3^a \pm 0.02$
N-retention (g/bird/d)	$1.2^a \pm 0.34$	$1.5^a \pm 0.18$	$1.6^a \pm 0.02$	$1.9^a \pm 0.19$

a,b, :Means in the same row not sharing a common superscript are significantly different (P<0.05)

\# :UNZ_0: 0 g of Natuzyme50®/kg DM feed; $UNZ_{0.75}$: 0.75g of Natuzyme50®/kg DM feed; $UNZ_{1.0}$: 1.0g of Natuzyme50®/kg DM feed; $UNZ_{1.5}$: 1.50g of Natuzyme50®/kg DM feed.

* : Values presented as mean ± standard error (SE)

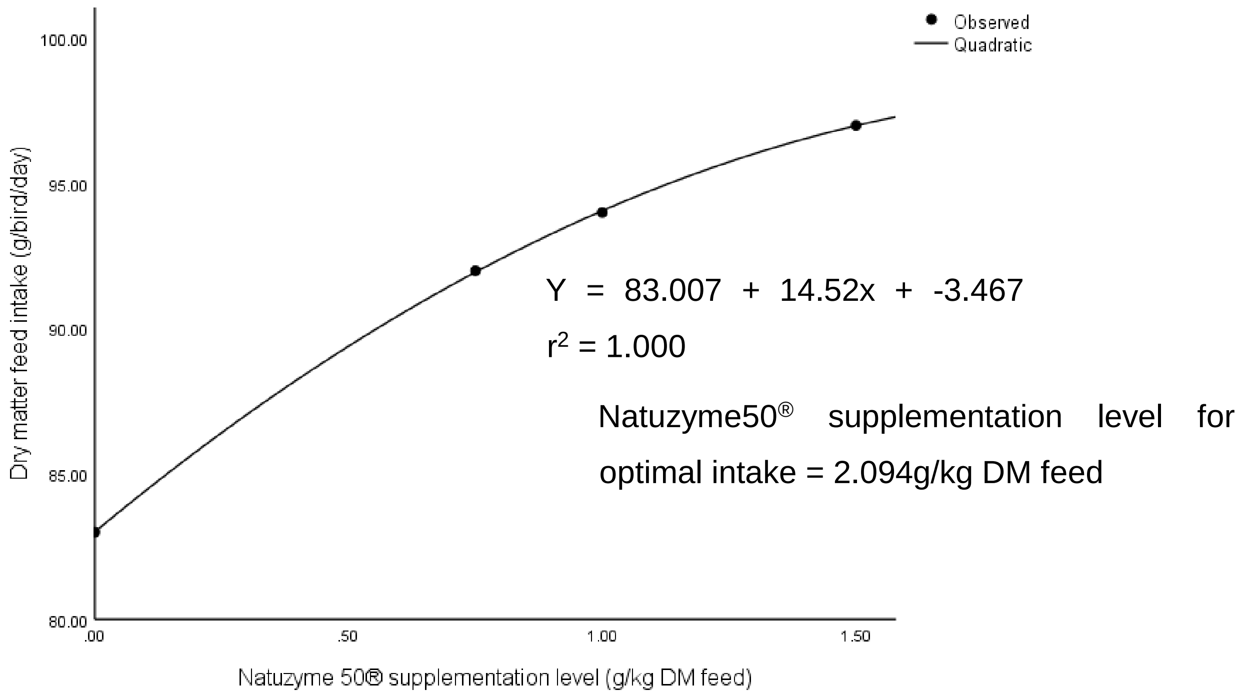

Figure 4.01 Effect of natuzyme50® supplementation level on dry matter intake of unsexed Ross 308 broiler chickens aged one to 21 days

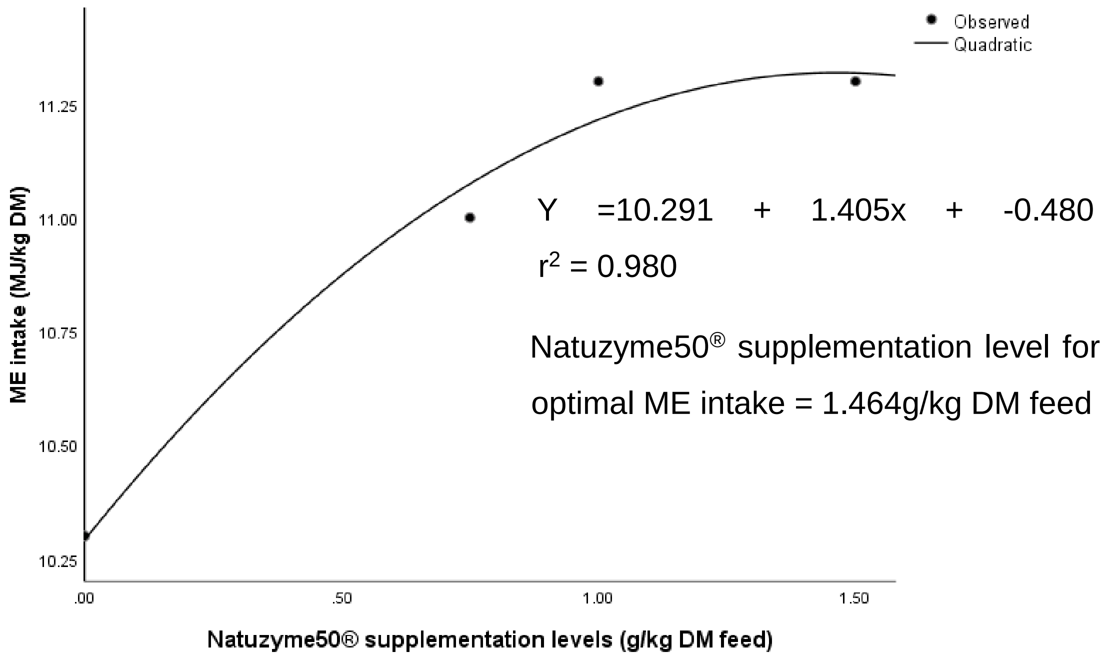

Figure 4.02 Effect of natuzyme50® supplementation level on ME intake of unsexed Ross 308 broiler chickens aged one to 21

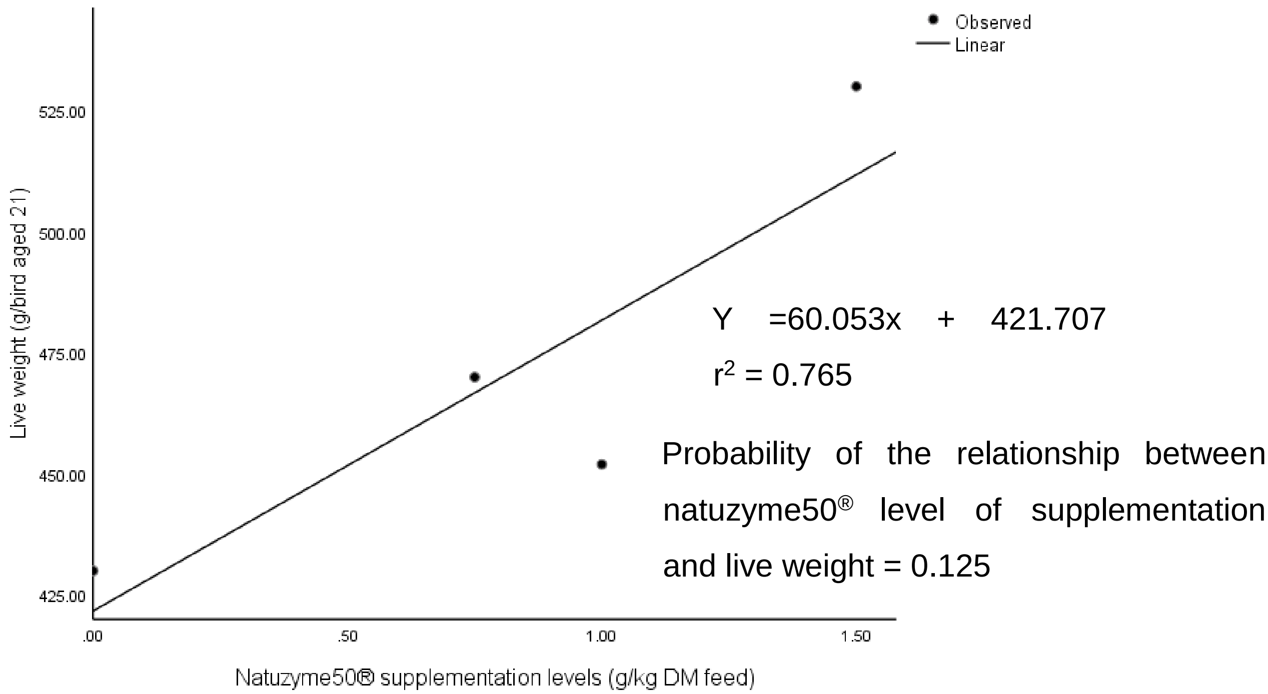

Figure 4.03 Relationship between natuzyme50® supplementation level and live weight of unsexed Ross 308 broiler chickens aged 21 days

Table 4.03 Natuzyme50® supplementation levels for optimal DM feed intake (g/bird/day) and ME intake (MJ/kg DM) of unsexed Ross 308 broiler chickens aged one to 21 days

Trait	Formula	NZ Level	Optimal Y-value	r^2
DM Intake	Y = 83.007 + 14.52x +- 3.467x^2	2.094	98.210	1.000
ME intake	Y = 10.291 + 1.405x +-0.480x^2	1.464	4.918	0.980

NZ level: Natuzyme50® supplementation level for optimal Y-value

r^2 : coefficient of determination

4.2 Male Ross 308 broiler chickens aged 22 to 42 days (Experiment 2)

Results of the nutrient composition of the diets for Experiment 2 are presented in Table 4.04. The diets had similar protein level of 20% and energy content of 16.92MJ/kg DM. The diets had different natuzyme50® supplementation levels of 0.0, 0.75, 1.0 and 1.5g/kg DM feed.

Results of the effect of natuzyme50® supplementation level on feed intake, growth rate, live weight, feed conversion ratio, metabolisable energy intake and N-retention of male Ross 308 broiler chickens aged 22 to 42 days are presented in Table 4.05. Natuzyme50® supplementation level had no effect (P>0.05) on DM intake of male Ross 308 broiler chickens aged 22 to 42 days. However, natuzyme50® supplementation level had effect (P<0.05) on growth rate, live weight, feed conversion ratio, metabolisable energy intake and N-retention of male Ross 308 broiler chickens. Male Ross 308 broiler chickens on a diet supplemented with 1.5g of natuzyme50® per kg DM had higher (P<0.05) growth rates than those on a diet supplemented with 1.0g of natuzyme50® per kg DM. However, chickens on a diet not supplemented with natuzyme50® or on diets supplemented with 0.75 or 1.5g of natuzyme50® per kg DM had similar (P>0.05) growth rates. Similarly, chickens on a diet not supplemented with natuzyme50® or on a diet supplemented with 1.0g of natuzyme50® per kg DM had the similar (P>0.05) growth rates.

Table 4.04 Diet composition (% except MJ/kg DM for energy and mg/kg DM for Zn, Cu, MN and Fe) for Experiment 2

Feed Nutrient	Treatment#			
	MNZ_0	$MNZ_{0.75}$	$MNZ_{1.0}$	$MNZ_{1.5}$
DM	91	91	91	91
CP	20	20	20	20
Energy	16.92	16.92	16.92	16.92
ADF	7.58	7.58	7.58	7.58
NDF	21.13	21.91	21.91	21.13
Fat	3.47	3.47	3.47	3.47
Ash	6.57	6.57	6.57	6.57
Ca	1.0	1.0	1.0	1.0
Mg	0.24	0.24	0.24	0.24
K	1.00	1.00	1.00	1.00
Na	0.14	0.14	0.14	0.14
K/Ca+Mg	0.47	0.47	0.47	0.47
P	0.81	0.81	0.81	0.81
Zn	54	54	54	54
Cu	12	12	12	12
Mn	144	144	144	144
Fe	327	327	327	327
NZ	0	0.75	1.0	1.5

:MNZ_0: 0 g of Natuzyme50®/kg DM feed; $MNZ_{0.75}$: 0.75g of Natuzyme50®/kg DM feed; $MNZ_{1.0}$: 1.0g of Natuzyme50®/kg DM feed; $MNZ_{1.5}$: 1.50g of Natuzyme50®/kg DM feed.

Live weights of male Ross 308 broiler chickens aged 42 days were affected ($P<0.05$) by natuzyme50® supplementation level (Table 4.05). Male Ross 308 broiler chickens on a diet supplemented with 1.5g of natuzyme50® per kg DM had higher ($P<0.05$) live weights than those on a diet supplemented with 1.0g of natuzyme50® per kg DM. However, male chickens on a diet not supplemented with natuzyme50® or on diets supplemented with 0.75 or 1.5g of natuzyme50® per kg DM had similar (P>0.05) live weights. Similarly, male chickens on a diet not supplemented with natuzyme50® or on a diet supplemented with 1.0g of natuzyme50® per kg DM had the same (P>0.05) live weights.

Table 4.05 Effect of natuzyme50® supplementation level on DM feed intake (g/bird/day), growth rate (g/bird/day), live weight (g/bird aged 42), feed conversion ratio (g DM feed/g live weight gain), metabolisable energy (ME) intake (MJ/kg DM) and nitrogen retention (g/bird/day) of male Ross 308 broiler chickens aged 22 to 42 days*

Variable	Treatment#			
	MNZ_0	$MNZ_{0.75}$	$MNZ_{1.0}$	$MNZ_{1.5}$
DM intake	$140^a \pm 6.6$	$130^a \pm 5.2$	$128^a \pm 2.6$	$127^a \pm 9.6$
Growth rate	$52^{ab} \pm 4.7$	$54^a \pm 3.5$	$38^b \pm 6.1$	$56^a \pm 3.7$
Live weight	$2044^{ab} \pm 117.0$	$2109^a \pm 108.9$	$1683^b \pm 172.1$	$2168^a \pm 90.8$
FCR	$2^b \pm 0.3$	$2^b \pm 0.1$	$3^a \pm 0.5$	$2^b \pm 0.3$
ME intakes	$12.5^b \pm 0.20$	$12.5^b \pm 0.20$	$13.3^a \pm 0.12$	$13.3^a \pm 0.12$
N-retention	$1.3^{bc} \pm 0.10$	$2.0^a \pm 0.07$	$1.2^c \pm 0.15$	$2.4^a \pm 0.30$

a,b, : Means in the same row not sharing a common superscript are significantly different (P<0.05).

: MNZ_0: 0 g of Natuzyme50®/kg DM feed; $MNZ_{0.75}$: 0.75g of Natuzyme50®/kg DM feed; $MNZ_{1.0}$: 1.0g of Natuzyme50®/kg DM feed; $MNZ_{1.5}$: 1.50g of Natuzyme50®/kg DM feed.

* : Values presented as mean ± standard error (SE)

Male Ross 308 broiler chickens on diets supplemented with 0.75 or 1.5g of natuzyme50® per kg DM and those without natuzyme50® supplementation had better FCR values (P<0.05) than those on a diet supplemented with 1.0g of natuzyme50® per kg DM (Table 4.05). However, chickens on a diet not supplemented with natuzyme50® or on diets supplemented with 0.75 or 1.5g of natuzyme50® per kg DM had similar (P>0.05) FCR values.

Metabolisable energy intakes of male Ross 308 broiler chickens aged 22 to 42 days were affected (P<0.05) by natuzyme50® supplementation level (Table 4.05). Male Ross 308 broiler chickens on diets supplemented with 1.0 or 1.5g of natuzyme50® per kg DM had higher (P<0.05) ME intakes than those on diet supplemented with 0.75g of natuzyme50® per kg DM or on a diet not supplemented with natuzyme50®. However, chickens on diets supplemented with 1.0 or 1.5g of natuzyme50® per kg DM had similar ME intakes (P>0.05). Similarly, chickens on a diet not supplemented with natuzyme50® or on a diet supplemented with 0.75g of natuzyme50® per kg DM had the same (P>0.05) ME intakes.

N-retention of male Ross 308 broiler chickens aged 22 to 42 days were affected (P<0.05) by natuzyme50® supplementation level (Table 4.05). Male Ross 308 broiler chickens on diets supplemented with 0.75 or 1.5g of natuzyme50® per kg DM had higher (P<0.05) N-retention than those on diet supplemented with 1.0g of natuzyme50® per kg DM or on a diet not supplemented with natuzyme50®. However, chickens on diets supplemented with 0.75 or 1.5g of natuzyme50® per kg DM had similar N-retention (P>0.05). Similarly, chickens on a diet not supplemented with natuzyme50® or on a diet supplemented with 1.0g of natuzyme50® per kg DM had the same (P>0.05) N-retention.

Results of the effect of natuzyme50® supplementation level on gut organ digesta pH values of male Ross 308 broiler chickens aged 42 days are presented in Table 4.06. Natuzyme50® supplementation had no effect (P>0.05) on crop, gizzard, proventriculus, ileum, caecum and large intestine digesta pH values of male Ross 308 broiler chickens aged 42 days.

Table 4.06 Effect of natuzyme50® supplementation level on gut organ digesta pH values of male Ross 308 broiler chickens aged 42 days*

	Treatment#			
Gut organ	MNZ_0	$MNZ_{0.75}$	$MNZ_{1.0}$	$MNZ_{1.5}$
Crop	$4.5^a \pm 0.15$	$4.4^a \pm 0.21$	$4.1^a \pm 0.07$	$4.0^a \pm 0.31$
Gizzard	$3.5^a \pm 0.26$	$3.1^a \pm 0.25$	$3.4^a \pm 0.09$	$3.5^a \pm 0.18$
Proventriculus	$4.4^a \pm 0.36$	$4.0^a \pm 0.16$	$4.4^a \pm 0.04$	$4.2^a \pm 0.04$
Ileum	$5.7^a \pm 0.10$	$5.4^a \pm 0.26$	$5.0^a \pm 0.24$	$5.5^a \pm 0.24$
Caecum	$6.4^a \pm 0.13$	$6.4^a \pm 0.04$	$6.5^a \pm 0.10$	$6.5^a \pm 0.03$
Large intestine	$5.5^a \pm 0.29$	$5.8^a \pm 0.10$	$5.6^a \pm 0.23$	$5.8^a \pm 0.12$

a : Means in the same row sharing a common superscript are significantly not different (P>0.05).

: MNZ_0: 0 g of Natuzyme50®/kg DM feed; $MNZ_{0.75}$: 0.75g of Natuzyme50®/kg DM feed; $MNZ_{1.0}$: 1.0g of Natuzyme50®/kg DM feed; $MNZ_{1.5}$: 1.50g of Natuzyme50®/kg DM feed.

* : Values presented as mean ± standard error (SE)

Results of the effect of natuzyme50® supplementation level on gut organ weights and lengths of male Ross 308 broiler chickens aged 42 days are presented in Table 4.07. Natuzyme50® supplementation did not (P>0.05) have any effect on crop, gizzard, caecum and liver weights of male Ross 308 broiler chickens aged 42 days. However, male Ross 308 broiler chickens on a diet supplemented with 1.5g of natuzyme50® per kg DM had higher (P<0.05) proventriculus weights than those on a diet supplemented with 1.0g of natuzyme50® per kg DM.

Chickens on a diet not supplemented with natuzyme50® or on diets supplemented with 1.5 or 0.75g of natuzyme50® per kg DM had similar (P>0.05) proventriculus weights. Similarly, chickens on a diet not supplemented with natuzyme50® or on diets supplemented with 0.75 or 1.0g of natuzyme50® per kg of DM had the same (P>0.05) proventriculus weights.

Table 4.07 Effect of natuzyme50® supplementation level on gut organ (g) weight and length (cm) of male Ross 308 broiler chickens aged 42 days*

	Treatment#			
Gut organ	MNZ_0	$MNZ_{0.75}$	$MNZ_{1.0}$	$MNZ_{1.5}$
Organ weight (g)				
Crop	$29^{a} \pm 0.5$	$32^{a} \pm 0.7$	$29^{a} \pm 1.5$	$35^{a} \pm 3.9$
Gizzard	$8^{a} \pm 0.6$	$11^{a} \pm 0.8$	$8^{a} \pm 0.4$	$10^{a} \pm 1.9$
Proventriculus	$49^{ab} \pm 2.1$	$58^{ab} \pm 0.6$	$47^{b} \pm 2.6$	$71^{a} \pm 11.2$
Small Intestine	$3^{ab} \pm 0.3$	$2^{b} \pm 0.1$	$3^{ab} \pm 0.7$	$4^{a} \pm 0.5$
Caecum	$10^{a} \pm 1.4$	$10^{a} \pm 1.5$	$8^{a} \pm 0.7$	$8^{a} \pm 0.1$
Large intestine	$38^{b} \pm 1.5$	$46^{a} \pm 0.7$	$40^{ab} \pm 3.2$	$46^{a} \pm 1.6$
Liver	$7^{a} \pm 0.0$	$8^{a} \pm 1.6$	$8^{a} \pm 0.5$	$9^{a} \pm 0.8$
Organ length (cm)				
Whole GIT	$206^{a} \pm 10.4$	$194^{a} \pm 6.6$	$212^{a} \pm 6.7$	$220^{a} \pm 12.3$
Small intestine	$193^{a} \pm 9.9$	$184^{a} \pm 6.0$	$201^{a} \pm 6.5$	$208^{a} \pm 11.9$
Caecum	$19^{a} \pm 1.3$	$18^{a} \pm 0.4$	$16^{a} \pm 1.3$	$17^{a} \pm 1.3$
Large intestine	$11^{a} \pm 0.8$	$11^{a} \pm 0.4$	$11^{a} \pm 0.3$	$11^{a} \pm 0.4$

a, b, : Means in the same row not sharing a common superscript are significantly different (P<0.05).

: MNZ_0: 0 g of Natuzyme50®/kg DM feed; $MNZ_{0.75}$: 0.75g of Natuzyme50®/kg DM feed; $MNZ_{1.0}$: 1.0g of Natuzyme50®/kg DM feed; $MNZ_{1.5}$: 1.50g of Natuzyme50®/kg DM feed.

* : Values presented as mean ± standard error (SE)

Small intestine weights of male Ross 308 broiler chickens were affected (P<0.05) by natuzyme50® supplementation level (Table 4.07). Male Ross 308 broiler chickens fed a diet supplemented with 1.5g of natuzyme50® per kg of DM had higher (P<0.05) small intestine weights than those on a diet supplemented with 0.75g of natuzyme50® per kg DM. However, chickens on a diet not supplemented with natuzyme50® or on diets supplemented with 1.0g or 1.5g of natuzyme50® per kg of DM had similar (P>0.05) small intestine weights. Similarly, chickens on a diet not supplemented with natuzyme50® or on diets supplemented with 0.75 or 1.0g of natuzyme50® per kg DM had the same (P>0.05) small intestine weights.

Male Ross 308 broiler chickens on a diet supplemented with 1.5g of natuzyme50® per kg DM had higher (P<0.05) large intestine weights than those on a diet not supplemented with natuzyme50® (Table 4.07). However, male Ross 308 broiler chickens on diets supplemented with 0.75, 1.0 or 1.5g of natuzyme50® per kg DM had similar (P>0.05) large intestinal weights. Similarly, chickens on a diet not supplemented with natuzyme50® or on a diet supplemented with 1.0g of natuzyme50® per kg DM had the same (P>0.05) large intestine weights (Table 4.07). Natuzyme50® supplementation level had no effect (P>0.05) on the lengths of gut organs of male Ross 308 broiler chickens aged 42 days (Table 4.07).

Results of the effect of natuzyme50® supplementation level on body and carcass weights of male Ross 308 broiler chickens aged 42 days are presented in Table 4.08. Natuzyme50® supplementation level had no effect (P>0.05) on carcass, breast and drumstick weights of male Ross 308 broiler chickens. Live weights of male Ross broiler chickens aged 42 days were affected (P<0.05) by natuzyme50® supplementation levels.

Male Ross 308 broiler chickens on a diet supplemented with 1.5g of natuzyme50® per kg DM had higher (P<0.05) live weights than those on a diet supplemented with 1.0g of natuzyme50® per kg DM. However, male Ross 308 broiler chickens on a diet not supplemented with natuzyme50® or on diets supplemented with 0.75 or 1.5g of natuzyme50® per kg DM had similar (P>0.05) live weights. Similarly, chickens on a diet not supplemented with natuzyme50® or on a diet supplemented with 1.0g of natuzyme50® per kg DM had the same (P>0.05) live weights at the age of 42 days.

Thigh weights of male Ross 308 broiler chickens aged 42 days were affected (P<0.05) by natuzyme50® supplementation levels (Table 4.08). Broiler chickens on a diet supplemented with 0.75g of natuzyme50® per kg DM had higher ($P<0.05$) thigh weights than those on a diet supplemented with 1.0g of natuzyme50® per kg DM. However, male Ross 308 broiler chickens on a diet not supplemented with natuzyme50® or on a diet supplemented with 0.75g of natuzyme50® per kg had similar ($P>0.05$) thigh weights. Similarly, chickens on a diet not supplemented with natuzyme50® or on diets supplemented with 0.75g, 1.0 or 1.5g of natuzyme50® per kg DM had the same ($P>0.05$) thigh weights (Table 4.08).

Table 4.08 Effect of natuzyme50® supplementation level on carcass, breast, drumstick and thigh weights (g) of male Ross 308 broiler chickens aged 42 days*

Variable	Treatment#			
	MNZ_0	$MNZ_{0.75}$	$MNZ_{1.0}$	$MNZ_{1.5}$
Live weight	$2044^{ab} \pm 117.0$	$2109^{a} \pm 108.8$	$1683^{b} \pm 172.1$	$2168^{a} \pm 90.8$
Carcass weight	$1120^{a} \pm 117.0$	$1175^{a} \pm 108.9$	$989^{a} \pm 172.1$	$1240^{a} \pm 90.8$
Breast weight	$369^{a} \pm 85.5$	$351^{a} \pm 91.1$	$308^{a} \pm 131.3$	$386^{a} \pm 71.8$
Drumstick weight	$145^{a} \pm 14.9$	$156^{a} \pm 12.2$	$140^{a} \pm 18.0$	$161^{a} \pm 10.7$
Thigh weight	$169^{ab} \pm 10.0$	$182^{ab} \pm 13.9$	$146^{b} \pm 17.0$	$146^{b} \pm 17.0$

a, b, : Means in the same row not sharing a common superscript are significantly different ($p<0.05$)

\# : MNZ_0: 0 g of Natuzyme50®/kg DM feed; $MNZ_{0.75}$: 0.75g of Natuzyme50®/kg DM feed; $MNZ_{1.0}$: 1.0g of Natuzyme50®/kg DM feed; $MNZ_{1.5}$: 1.50g of Natuzyme50®/kg DM feed.

* : Values presented as mean ± standard error (SE)

Results of the effect of natuzyme50® supplementation level on meat tenderness, juiciness, flavour and shear force for male Ross 308 broiler chickens aged 42 days are presented in Table 4.09. Natuzyme50® supplementation levels had no effect ($P>0.05$) on the Juiciness of male Ross 308 broiler chicken meat. However, natuzyme50® supplementation level affected ($P<0.05$) broiler chicken meat tenderness. Chickens on diets supplemented with 1.5g natuzyme50® per kg DM had higher ($P<0.05$) meat tenderness values than those on diets not supplemented with natuzyme50®. However, meat from chickens on diets supplemented with 0.75, 1.0 or 1.5g of natuzyme50® per kg of DM had similar (P>0.05) tenderness values. Similarly, chickens on a diet not supplemented with natuzyme50® or on diets supplemented with 0.75 or 1.0g of natuzyme50® per kg DM had same (P>0.05) meat tenderness values.

Table 4.09 Effect of natuzyme50® supplementation level on meat tenderness, juiciness, flavour and shear force values for male Ross 308 broiler chickens aged 42 days*

Variable	Treatment#			
	MNZ_0	$MNZ_{0.75}$	$MNZ_{1.0}$	$MNZ_{1.5}$
Tenderness	2.7^b±0.18	3.2^{ab} ± 0.17	2.9^{ab} ± 0.26	3.7^a ± 0.03
Juiciness	2.6^a ± 0.24	3.2^a ± o.43	2.8^a ± 0.09	3.3^a ± 0.20
Flavour	3.2^b ± 0.08	3.3^{ab} ± 0.06	3.4^{ab} ± 0.03	3.5^{ab} ± 0.15
Shear force	20^a ± 1.6	15^{ab} ± 1.4	14^{ab} ± 0.7	11^b ± 1.5

a, b, : Means in the same row not sharing a common superscript are significantly different (P<0.05)

: MNZ_0: 0 g of Natuzyme50®/kg DM feed; $MNZ_{0.75}$: 0.75g of Natuzyme50®/kg DM feed; $MNZ_{1.0}$: 1.0g of Natuzyme50®/kg DM feed; $MNZ_{1.5}$: 1.50g of Natuzyme50®/kg DM feed

* : Values presented as mean ± standard error (SE)

Meat flavour of male Ross 308 broiler chickens was affected (P<0.05) by natuzyme50® supplementation level (Table 4.09). Meat of chickens on a diet supplemented with 1.5g of natuzyme50® per kg DM had better flavour than meat from chickens on a diet not supplemented with natuzyme50®. However, meat from chickens on diets supplemented with 0.75, 1.0 or 1.5g of natuzyme50® per kg DM had similar (P>0.05) flavour. Similarly, meat from chickens on a diet not supplemented with natuzyme50® or on diets supplemented with 0.75 or 1.0g of natuzyme50® per kg DM had the same (P>0.05) flavour.

Natuzyme50® supplementation level affected (P<0.05) chicken meat shear force values (Table 4.09). Meat from male Ross 308 chickens on a diet not supplemented with natuzyme50® had higher (P<0.05) meat shear force values than meat from chickens on a diet supplemented with 0.75g of natuzyme50® per kg DM. However, meat from chickens on a diet not supplemented with natuzyme50® or on diets supplemented with 0.75 or 1.0g of natuzyme50® per kg DM had similar (P>0.05) shear force values. Similarly, meat from chickens on a diet not supplemented with natuzyme50® or on diets supplemented with 0.75 or 1.0g of natuzyme50® per kg DM had the same (P>0.05) shear force values.

Positive relationships were observed between natuzyme50® supplementation levels and meat tenderness (r = 0.851) and flavour (r = 0.963) of male Ross 308 broiler chickens aged 42 days (Figures 4.04 and 4.05, respectively and Table 4.09). However, a negative relationship was observed between natuzyme50® supplementation levels and meat shear force values of male Ross 308 broiler chickens aged 42 days (Figure 4.06 and Table 4.09)

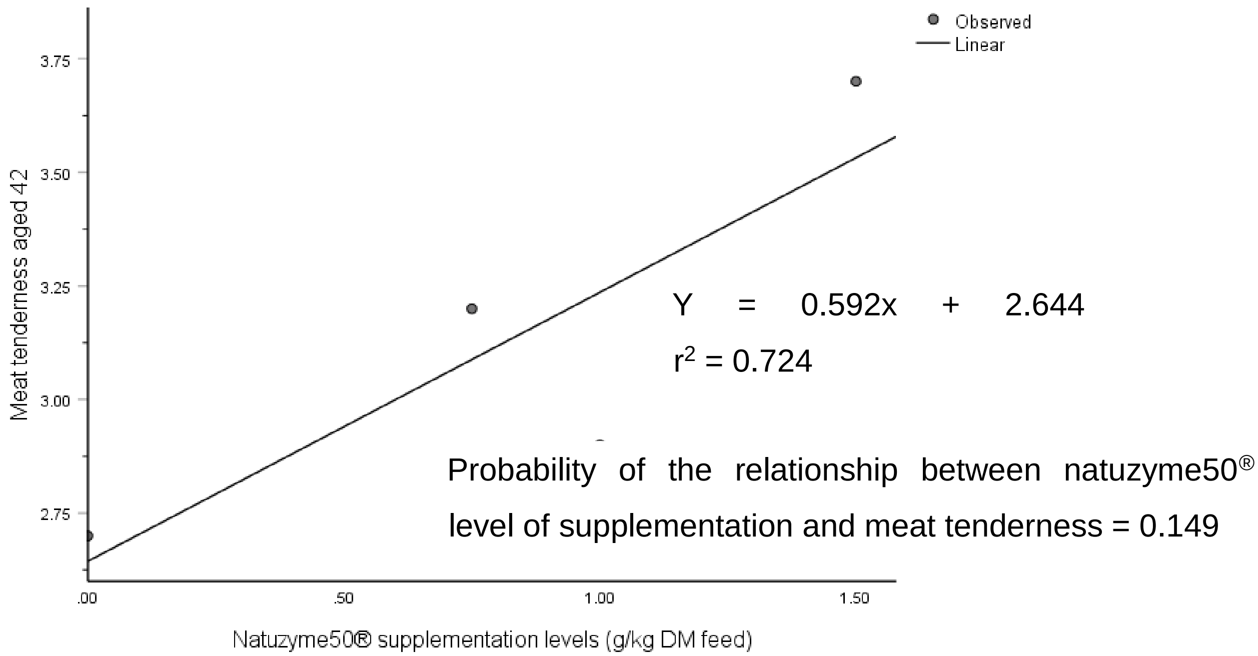

Figure 4.04 Effect of natuzyme50® supplementation on meat tenderness of male Ross 308 broiler chickens aged 42 days

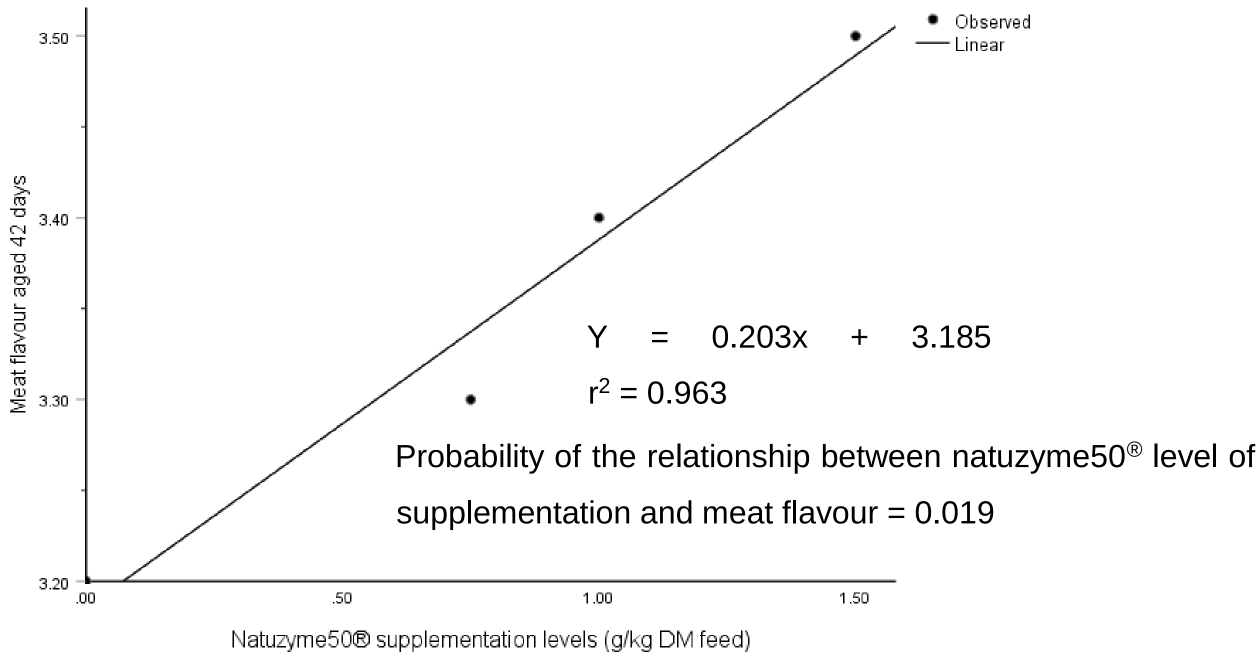

Figure 4.05 Effect of natuzyme50® supplementation on meat flavour of male Ross 308 broiler chicken aged 42 days

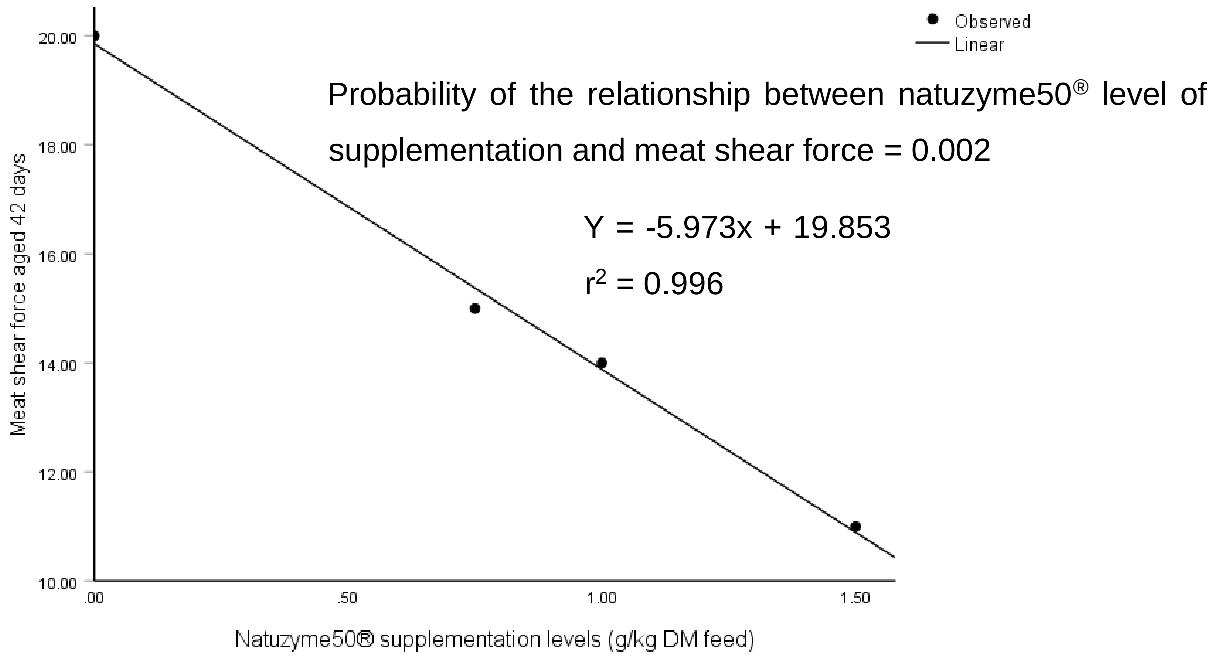

Figure 4.06 Effect of natuzyme50® supplementation on meat shear force value for male Ross 308 broiler chickens aged 42 days

CHAPTER 5

DISSCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

5.1.1 Unsexed Ross 308 broiler chickens aged one to 21 days

The diets in the present study were formulated to have crude protein, energy and crude fibre levels of 21%, 16MJ/kg DM and 5%, respectively. The diets contained the same nutrients except for the natuzyme50® supplementation levels which ranged from 0 to 1.5g/kg DM. Nutrient composition of the diets were not changed by the natuzyme50® supplementation levels. The diets met the nutrient requirements for unsexed broiler chickens as specified by McDonald *et al.* (2010).

The results of the present study showed that supplementing diets with natuzyme50® improved feed intakes of unsexed Ross 308 broiler chickens aged one to 21 days (Figure 4.01). These findings are in line with the previous study done by Sharifi *et al.* (2013) who reported a significant increase in feed intake when supplementing the diet with natuzymes plus at a level of 0.035g/kg DM. Baghban-Kanani *et al.* (2018), Milanloo *et al.* (2012), Yaghobfar *et al.* (2008) and Berwal *et al.* (2008) also observed improved feed intakes when supplementing the diets with natuzyme. However, the present study is in contrast to the reports of Amao (2018), Oliaei *et al.* (2016) and Ahmadi (2016) who found no differences in feed intakes of broiler chickens fed diets supplemented with different levels of natuzyme50®. Fasiullah *et al.* (2010) observed decreased feed intake when the level of enzymes increased in the diets. Quadratic equations indicated that natuzyme50® supplementation level of 2.09g/kg DM would support optimal diet DM intake of unsexed Ross 308 broiler chickens aged one to 21 days. Makinde *et al.* (2013) concluded that addition of natuzyme enzyme, generally, improved intake of broiler chicken diets.

Natuzyme50® supplementation levels used in the present study did not affect growth of unsexed Ross 308 broiler chickens aged one to 21days. The results of the present study are in agreement with the findings of Mohammed *et al.* (2018), Fasiullah *et al.* (2010) and Seifi (2013) who did not observe any improvement in growth rate of broiler chickens fed diets supplemented with natuzyme. However, the results of the present study contradict with the report of Sharifi *et al.* (2013) who reported improved growth rates of chickens fed natuzymes supplemented to corn-wheat soybean or corn-wheat soybean canola diets. Oliaei *et a.l* (2016), Zaghari (2008) and Yaghobfar *et al.* (2008) reported an improved weight gain in broiler chickens fed diets supplemented with natuzyme at starter and rearing periods. The results of Yaghobfar *et al.* (2015) showed that natuzyme plus improved energy and protein efficiency for weight gain at a supplementation level of 0.350g/kg DM. Similar results were, also, observed by Milanloo *et al. (*2007) who reported an improved weight gain in broiler chickens fed diets supplemented with natuzyme at starter and rearing periods while Yaghobfar (2008) reported an improved weight gain at the starter period. Sherif (2009) declared that the diet supplemented with natuzyme had a significant positive effect on final body weight of chicks compared to diets without enzymes. The inclusion of natuzyme in broiler chicken diets increased live weight gain in broiler chickens by eliminating anti-nutritional factors contained in cereal diets and improving the utilization of dietary energy which in return improved broiler chicken performance (Ranjan *et al.,* 2018; Kalantar *et al.,* 2014; Ramesh *et al.,* 2006). The inconsistency in the findings may be due to application dosage of enzymes and chicken breeds used (Cowieson *et al*., 2006). Castillo and Gatlin (2014) concluded that there are possibilities for improved nutrient utilization that are not accompanied by increased growth rate.

Natuzyme50® supplementation levels used in the present study did not have any effect on feed conversion ratio of unsexed Ross 308 broiler chickens aged one to 21days. This may be attributed to the fact that natuzyme50® supplementation levels used did not affect nitrogen retention. This is similar to the findings of Amao (2018), Alefzadeh *et al.* (2016) and and Fasiullah *et al* (2010) who did not observe any significant changes on feed conversion ratio in broiler chickens that were fed natuzyme supplemented diets. Similarly, Makinde *et al.* (2014) reported no differences in FCR of broiler chickens fed diets supplemented with natuzyme. However, the present results are contrary to the findings of Mohammed *et al.* (2018) and Sharifi *et al.* (2013) who observed better FCR values on diets supplemented with natuzyme for chickens aged 7 to14 days. The authors suggested that improvement in FCR was due to improved diet digestibility.

Natuzyme50® supplementation levels used in the present study affected live weights of unsexed Ross 308 broiler chickens aged 21 days. Increasing natuzyme50®

supplementation level to 1.5 g/kg DM improved live weights of unsexed broiler chickens aged one to 21 days. These results are in agreement with those of Amao (2018) and Oliaei (2016) who reported that supplementing diets with natuzyme50® improved broiler chicken live weights.

The present study indicates that natuzyme50® supplementation levels affected metabolisable energy intakes of unsexed Ross 308 broiler chickens aged one to 21 days. Metabolisable energy intakes were optimized at a natuzyme50® supplementation level of 1.464g/kg DM feed. These results study are similar to the observation made by Yaghobfar *et al.* (2015), Yaghobfar (2008) and Olukosi *et al.* (2007) who reported increased ME intake values in broiler chickens when fed diets supplemented with natuzyme. These authors attributed the increase in ME intake to decreased gut viscosity and increased energy digestibility of the diets when natuzymes were supplemented.

5.1.2 Male Ross 308 broiler chickens aged 22 to 42 days

The diets in the present study were formulated to have crude protein, energy and crude fibre levels of 20%, 16.92 MJ/kg DM and 5%, respectively. The diets contained the same nutrients except for the natuzyme50® supplementation levels which ranged from 0 to 1.5g/kg DM. Nutrient composition of the diets were not changed by the natuzyme50® supplementation levels. The diets met the nutrient requirements for male broiler chickens as specified by McDonald *et al.* (2010).

Natuzyme50® supplementation levels used in the present study did not affect feed intake of male Ross 308 broiler chickens aged 22 to 42 days. The present results are similar to the findings of Taheri and Shirzadegan (2017), Abolade (2016), Lee *et al.* (2014), Seifi (2013) and Jalan *et al.* (2007) who did not observe any significant changes in feed intake of broiler chickens that were fed natuzyme-supplemented diets. Mirakzeni *at al.* (2010) reported similar feed intakes when chickens were fed natuzyme-supplemented diets. The results of Attamangkune *et al.* (2007) showed that the addition of natuzyme to feed containing descending levels of metabolisable energy (ME) did not affect feed intake and feed efficiency. Swain *et al.* (2014) and Zakaria *et al.* (2010) reported a decrease in feed intake by broiler chickens when diets were supplemented with natuzymes. However, the present results are contrary to the findings of Yaghobfar (2008) who observed improved feed intakes of broiler chickens fed diets supplemented with natuzyme. The authors attributed this to improved diet digestibility.

The results of the present study showed that supplementing diets with natuzyme50® did not improve growth rates of male Ross 308 broiler chickens aged 22 to 42 days. The present results are similar to those of Seifi (2013) who showed that addition of natuzyme to diets with 15 or 30% wheat had no significant effect on body weight gain of broiler chickens. Sarica *et al.* (2005) observed no differences in body weight gains of broiler chickens supplemented with different levels of natuzymes. These results are contrary to those of Taheri and Shirzadegan (2017), Oliaei (2016), Swain *et al*. (2014), Dehghani (2013) and Zaghari (2008) who reported improved body weight gains for similar natuzyme50® supplementation levels. These authors observed that natuzyme50® hydrolyzed NSP, reduced digesta viscosity, and improved nutrient absorption and growth performance. Alefzadeh *et al*. (2016), also, observed improved live weight gains but with no effect on FCR for broiler chickens that were fed enzyme-supplemented diets. Zamini *et al*. (2014) stated that the use of natuzyme is beneficial for growth. This is similar to the study of Larhang and Torki (2011) who reported improvement on body weight and body weight gain of chickens fed diets with natuzyme. Makinde *et al.* (2018) suggested that rice offal-contained diets supplemented with natuzyme can be used as an alternative source of energy in the finisher diets for broiler chickens without adverse effects on growth performance.

Natuzyme50® supplementation levels used in the present study did not improve live weights of male Ross 308 broiler chickens aged 42 days. The present results are in agreement with the findings of Seifi (2013) and Fasiullah *et al*. (2010) who reported no improvements in live weight gain of broiler chickens fed diets supplemented with natuzyme. Mohammed *et al.* (2018) did not observe any effects on the live weight of broiler chickens fed natuzyme. On the other hand, the present results contradict with those of Alabi *et al.* (2017) and Yaghobfar *et al.* (2008) who reported higher weight gains of broiler chickens fed wheat bran-based diets supplemented with natuzyme and maxigrain. Larhang and Torki (2011) reported that inclusion of different levels of guar meal (0.4 and 8%) and commercial energy (0 and 0.05% of natuzyme) resulted in highest body weight and body weight gains. Swain *et al.* (2014) reported improved body weight when supplementing natuzyme50® at levels ranging from 1.0 to 1.5g/kg of DM of feed. This is similar to the results of Dehghani (2013), Cowieson and Ravindran (2008[b]) and Gao *et al*. (2007) who observed that adding enzymes to the diet improved live weights of broiler chickens.

The present study showed that natuzyme50® supplementation levels used did not improve FCR of male Ross 308 broiler chickens aged 22 to 42 days. These findings are similar to those of Seifi (2013), Malakzadegan *et al*. (2012) and Fasiullah *et al.* (2010) who did not observe any improvement in FCR values of broiler chickens fed diets supplemented with natuzymes. Makinde *et al.* (2014) did not observe differences across the treatment groups of broiler chickens when the diets were supplemented with natuzymes. Horvatovic *et al.* (2014) indicated that although enzyme supplementation reduced digesta viscosity in the finisher phase, no any improvement were found in FCR values. Zeng *et al.* (2015), Brufau *et al.* (2006), Scheideler *et al.* (2005) and Soliman (1997) reported no differences in feed conversion ratio of broiler chickens fed enzyme supplemented diets. The present results are contradictory to the observations made by Amerah *et al.* (2017), Ahmadi (2016), Chotinsky (2015) and Saleh *et al.* (2005) who indicated that adding enzymes to the diets improved FCR values. Horvatovic *et al*. (2015) and Mohammed *et al.* (2010) also reported better broiler chicken FCR values with natuzyme supplementation to the grower diets. Nikam *et al.* (2016) concluded that supplementing NSP hydrolysing enzymes to corn soyabean meal-based diets improved FCR values of broiler chickens reared on sub-optimal diets.

The results of the present study showed that supplementing diets with natuzyme50® at a level of 1.0 and 1.5g/kg DM improved ME intakes of male Ross 308 broiler chickens. This findings are in agreement with Yaghobfar (2014) who reported improved ME intakes when natuzyme plus was supplemented at a level of 0.350 g/kg DM diet. Oukosi *et al.* (2008) observed improved metabolisable energy (ME) in broiler chickens diets supplemented with enzymes. The results of the present study are in contrast to the findings of Mujeeb (2007) that the addition of 250 or 500 ppm of natuzyme did not affect ME intake and feed conversion ratio values of broiler chickens.

The present study showed that natuzyme50® supplementation levels used improved N-retention of male Ross 308 broiler chickens aged 42 days. Supplemented diets with natuzyme50® at a level of 0.75 and 1.5g/kg DM improved N-retention of male Ross 308 broiler chickens. These findings are similar to those of Oukosi *et al* (2008) and Alagawany (2017) who reported an improved N-retention of broiler chickens. Sherif (2009) reported that the addition of natuzyme in the broiler diet led to significant improvement in Nitrogen retention rate compared to the control group. The present results are contradictory to the observations made by Zaghari *et al* (2008) who did not observe any significant effect of N-retention in broiler chickens fed diets supplemented with natuzyme

Natuzyme50® supplementation levels used in the present study did not affect gut organ digesta pH values of male Ross broiler chickens aged 42 days. This is similar to the study of Ravindran (2013) who did not observe any effect on gut organ digesta pH values when broiler chickens were fed diets supplemented with natuzymes. Suhermiyati and Iriyanti (2013) indicated that natuzyme works optimally at pH 5. Mohammed (2018) reported significantly lower gut organ digesta pH values in chickens on diets containing 1kg of natuzyme50 plus per tonne DM.

Natuzyme50® supplementation levels used in the present study did not affect of crop, gizzard, caecum and liver weights of broiler chickens aged 42 days. This is similar to the studies of Mahmood *et al.* (2017) and Alefzadeh *et al.* (2016) who reported no significant effect on gut weight segments of broiler chickens fed natuzyme- supplemented diets. Taheri and Shirzadegan (2017), Lee *at al.* (2014) and Makinde *et al.* (2014) observed no differences in broiler chicken liver weights with natuzyme supplementation. Sarica *et al.* (2005), also, observed no differences in liver weights when broiler chickens were supplemented with natuzymes. Milanloo *et al.* (2012) reported a reduced broiler chicken liver weight when natuzyme plus was supplemented to a diet containing wheat and canola meal. The authors concluded that gut organ weights were not affected by natuzyme50® supplementation levels used in their study. However, the present results are contrary to the finding of Swain *et al.* (2014) and Amao (2018) who reported significant increases in the relative broiler chicken weights of gizzards, livers and intestines following dietary supplementation with natuzyme50® at levels ranging from 1.0 to 1.5g/kg DM of feed. Furthermore, Oliaei *et al.* (2016) reported improved broiler chicken liver weights with natuzyme50® supplementation.

The results of the present study showed that supplementing diets with natuzyme50® improved weights of proventriculus, small intestines and large intestines of male Ross 308 broiler chicken aged 42 days. A natuzyme50® supplementation level of 1.5 g/kg DM had higher broiler chicken proventriculus, small intestine and large intestine weights. This may be attributed to increase in ME intake with natuzyme50® supplementation. The findings of the current study are consistent with the study of Sharifi (2013) who reported an improved morphology of the small intestines in broiler chickens fed diets supplemented with natuzymes. Similar results were observed by Alabi *et al.* (2017) who reported an improved gizzard weight of broiler chickens fed diets supplemented with natuzyme, However the present results contrary to those of Alefzadeh *et al.* (2016) who observed no significant effect of natuzyme supplementation on these traits. Milanloo *et al.* (2012) and Yaghobfar *et al.* (2008) reported reduced relative weight of digesta organs in broiler chickens fed natuzyme- supplemented diets.

Natuzyme50® supplementation levels used in the present study did not affect lengths of GIT, small intestines, caecum and large intestines of male Ross 308 broiler chicken aged 42 days. These results are in agreement with the findings of Oliaei *et al.* (2016) who observed no effect of natuzyme supplementation on the lengths of gut segments. The results contradict with the report of Sharifi (2013) who observed improved small intestine lengths of broiler chickens fed diets supplemented with natuzyme.

Natuzyme50® supplementation levels used in the present study did not affect carcass, breast and drumstick weights of male Ross 308 broiler chickens aged 42 days. This is similar to the findings of Taheri and Shirzadegan (2017) and Alefzadeh *et al.* (2016) who did not observe any significant improvements in carcass, breast and drum stick weights of broiler chickens that were fed natuzyme50® supplemented diets. Similarly, Mohammed *et al.* (2018) did not observe any significant improvements in carcass and drumstick weights of broiler chickens supplemented with natuzymes. Zanella *et al.* (1999), also, reported no effect on carcass and breast meat weights when exogenous enzyme combination of xylanase, amalyse and phytase were fed to broiler chickens aged 42 days. The present study observed that the inclusion of natuzyme50® did not affect carcass weight of broiler chickens. However, Omojola and Adesehinwa (2007) observed that enzyme supplementation improved carcass yield of broiler chickens. Similarly, Swain *et al.* (2014) and Ismail *et al.* (2011) observed improved carcass weights of broiler chickens fed natuzyme50® supplemented diets. Alabi *et al* (2017) reported improved carcass weights of broiler chickens fed wheat-based diets supplemented natuzyme.

Natuzyme50® supplementation levels used in the present study did not improve thigh weights of male Ross 308 broiler chickens aged 42 days. These results are in agreement with Mahmood *et al.* (2017) who did not observe significant effects on thigh weights of broiler chickens fed natuzyme-supplemented diets. The present results are contradictory to the observations made by Ajayi and Akoma (2017) who reported improved thigh weights in broiler chickens fed diets supplemented with exogenous enzyme-protease. The authors attributed this to the increase in growth rate with natuzyme supplementation.

The natuzyme50® supplementation levels used in the present study had no effect on meat juiceness. However, natuzyme supplementation improved meat tenderness and flavour. The present study are in agreement with those of Liu *et al*. (2012) and Loddi *et al.* (2000) who reported that supplementing diets with natuzyme50® improved chicken meat tenderness and flavour. The authors attributed the improved meat tenderness and flavour to improved nutrient intake and absorption due to natuzyme50® supplementation. The results of the present study are contrary to those of Brzoska *et al.* (2010) who reported that supplementation with natuzyme50® did not have any effect on broiler chicken meat tenderness and flavour.

Increased natuzyme50® level of supplementation decreased meat shear forces values. This may be because natuzyme supplementation improved nutrient uptake (Amerah, 2015; Khan *et al.,* 2006).

5.2 CONCLUSIONS AND RECOMMENDATIONS

5.2.1 Unsexed Ross 308 broiler chickens aged one to 21 days

Natuzyme50® supplementation levels used in the present study improved feed intakes and live weights of unsexed Ross 308 broiler chickens aged one to 21 days. Natuzyme50® supplementation levels used did not affect growth rate, feed conversion ratio, metabolisable energy and N-retention of unsexed Ross 308 broiler chickens aged one to 21 days. However, various variables were improved at different natuzyme50® supplementation levels. This means dietary natuzyme50® supplementation levels for optimal productivity of the chickens will depend on the particular variable. These findings have a lot of implications on ration formulation for unsexed broiler chickens where natuzyme50® is used as a supplement. However, more studies should be done to ascertain the present responses.

5.2.2 Male Ross 308 broiler chickens aged 22 to 42 days

Natuzyme50® supplementation levels used in the present study affected growth rate, live weight, FCR, ME and N-retention of male Ross 308 broiler chickens aged 22 to 42 days. However, natuzyme50® supplementation level did not affect digesta pH values of crop, gizzard, proventriculus, ileum, caecum and large intestines of male Ross 308 broiler chickens aged 42 days. Similarly, natuzyme50® supplementation did not affect weights and lengths of crops, gizzards, livers, GTI, small intestines, caeca and large intestines of male broiler chickens. However, natuzyme50® supplementation levels used in the study affected proventriculus, small intestine and large intestine weights of male Ross 308 broiler chickens aged 42 days.

Natuzyme50® supplementation levels used in the present study had no effect on carcass weight, breast weight and drumstick weight of male broiler chickens. However, natuzyme50® supplementation levels used affected live weights and thigh weights of male broiler chickens. Natuzyme50® supplementation levels used in the present study improved broiler meat tenderness and flavour. Natuzyme50® supplementation, also, decreased meat shear force values. Thus, natuzyme50® supplementation may be very useful in improving broiler meat. Further studies are suggested.

CHAPTER 6

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