EFFECT OF SUPPLEMENTING DIETS WITH EFFECTIVE MICROORGANISMS ON INTAKE, GROWTH AND CARCASS CHARACTERISTICS OF ROSS 308 BROILER CHICKENS

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BY

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A MINI-DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN AGRICULTURE (ANIMAL PRODUCTION), DEPARTMENT OF AGRICULTURAL ECONOMICS AND ANIMAL PRODUCTION, SCHOOL OF AGRICULTURAL AND ENVIRONMENTAL SCIENCES, FACULTY OF SCIENCE AND AGRICULTURE, UNIVERSITY OF LIMPOPO, SOUTH AFRICA

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2014

DECLARATION

I declare that this mini-dissertation hereby submitted to the University of Limpopo for the degree of Master of Science in Agriculture (Animal Production) has not been submitted by me for a degree at this or any other University, this is my own work in design and execution, and that all materials contained herein has been duly acknowledged.

Signature…… Date…….

Miss Mafiri Maseko Nkele

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A special appreciation goes to my husband for his understanding, encouragement and support throughout the whole period of this study.

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DEDICATION

This dissertation is dedicated to my lovely mother, Mafiri Elisa Modjadji, my father Mafiri Sekhathale Daniel and my husband Mangwane Patrick Tintswalo, for their support during my study period.

ABSTRACT

Two experiments were conducted to determine the effects of supplementing diets with effective microorganisms on intake, growth and carcass characteristics of Ross 308 broiler chickens. The first experiment determined the effect of supplementing diets with effective microorganisms on performance of unsexed Ross 308 broiler chickens aged one to three weeks. Two hundred unsexed day-old chicks were randomly assigned to five treatments with four replications, each replication having ten birds. A complete randomized design was used. The chickens were fed a grower diet supplemented with 0 (EM_0), 30 (EM_{30}), 50 (EM_{50}), 70 (EM_{70}) or 100 (EM_{100}) ml of effective microorganisms per litre of water. Effective microorganism supplementation did not improve ($P>0.05$) diet and metabolisable energy intakes of the chickens. Effective microorganism supplementation reduced ($P<0.05$) growth rate and live weight of the chickens. Poorer ($P<0.05$) feed conversion ratios were observed in chickens supplemented with effective microorganisms. However, effective microorganism supplementation improved ($P<0.05$) crude protein retention of the chickens. Supplementation with effective microorganisms reduced ($P<0.05$) mortality rate of the chickens from 10 to 0 %.

The second experiment determined the effect of supplementing diets with effective microorganisms on performance of male Ross 308 broiler chickens aged 22 to 42 days. The chickens were randomly allocated to five treatments with four replications, each replication having 10 birds. The chickens aged 21 days, weighing 474 ± 2g, were allocated to the treatments in a complete randomized design. The chickens were fed a grower diet supplemented with 0 (EMM_0), 30 (EMM_{30}), 50 (EMM_{50}, 70 (EMM_{70}) or 100 (EMM_{100}) ml of effective microorganisms per litre of water. Effective microorganism supplementation did not improve ($P>0.05$) intake, DM digestibility, metabolisable energy, feed conversion ratio, fat pad weight and meat sensory attributes of the chickens. Effective microorganism supplementation reduced ($P<0.05$) growth rate, live weight and carcass weight of the chickens. However, effective microorganism supplementation improved ($P<0.05$) crude protein retention and crude protein content of meat of the chickens. Supplementation with effective microorganisms reduced ($P<0.05$) mortality of the chickens from 5 to 0 %.

It is concluded that effective microorganism supplementation to the diets of Ross 308 broiler chickens reduced growth rate and live weight of the chickens. However, effective microorganism supplementation improved crude protein retention and crude protein content of the meat of broiler chickens. Supplementation with effective microorganisms reduced mortality of the chickens to zero.

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CHAPTER 1

INTRODUCTION

1.1 Background

The broiler chicken industry is an important source of animal protein in South Africa (Boer *et al*., 2001). Small poultry holdings provide adequate protein, income and employment and contribute to poverty alleviation in South Africa (Sonaiya, 1999). Poultry meat provides an excellent source of protein, minerals and vitamins (Oduguwa *et al*., 2000). However, the increase in growth rate of modern broiler chickens has been associated with increased mortality and fat deposition (Plavnik *et al*., 1986). There is evidence that effective microorganism supplementation improves quality of broiler chickens (Cavazzoni *et al*., 1998). There is also some evidence that effective microorganism supplementation reduces mortality of chickens (Deiver 2008). However, other studies indicate that effective microorganism supplementation has no effect on growth and carcass characteristics of broiler chickens (Ergun *et al*., 2000). There is need, therefore, to ascertain these findings.

1.2 Problem statement

Broiler chicken production is financially, nutritionally and culturally important to the people of South Africa (Halima *et al*., 2009). Broiler chickens are characterized by very high growth rates and good feed conversion ratios (Tumova *et al*., 2002). However, incidences of metabolic diseases, low fibre digestion, high mortalities and increased carcass fat deposition tend to bring about economic losses to the industry (Hassanzadeh, 2009). There is some evidence that supplementing effective microorganisms to the diets of broiler chickens increases growth rate, and reduces mortality and fat deposition in broiler chickens (Ashraf *et al*., 2005). However, such information is not extensive and conclusive.

1.3 Motivation

This study will generate information on the effect of supplementing effective microorganisms to the diets on productivity and carcass characteristics of Ross 308 broiler chickens. Such information will help in the formulation of diets aimed at reducing mortality and fat deposition, and optimizing productivity of Ross 308 broiler chickens.

Optimization of productivity of the chickens will help to improve economic, nutritional and social status of broiler chicken farmers.

1.4 Objectives

The objectives of this study were as follows:

i. To determine the influence of supplementing diets with effective microorganisms on feed intake, digestibility, live weight, growth, feed conversion ratio, mortality and carcass characteristics of Ross 308 broiler chickens.

ii. To determine effective microorganism supplementation levels for optimal responses in feed intake, digestibility, live weight, growth, feed conversion ratio, mortality and carcass characteristics of Ross 308 broiler chickens.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Poultry meat is nutritionally and economically very important in the world. However, extensive genetic selection towards a fast-growing chicken has led not only to a dramatic shortening of the growing period, but also to excessive carcass fat, which consequently lowers meat yield and feed efficiency. There is a strong demand for meat products that are high in lean content and low in fat (Lippens *et al*., 2000). Similarly, broiler chickens have high mortality rates. There is some evidence indicating that supplementing effective microorganisms to the diets improves growth and reduces mortality and carcass fat of broiler chickens (Vicente *et al*., 2007). Effective microorganisms improved weight gain and feed utilization and reduced abdominal fat pads of broiler chickens (Patidar & Prajapati, 1999). However, in other studies (Panda *et al*., 2000) effective microorganism supplementation did not affect growth, mortality and fat content of broiler chickens. Effective microorganisms are mixed cultures of beneficial and naturally occurring microorganisms. They contain selected species of microorganisms including predominant populations of lactic acid bacteria, yeasts and smaller numbers of photosynthetic bacteria, actinomycetes and other types of organisms (Arshad, 2010).

2.2 Importance and biochemical functions of effective microorganisms

Each component of effective microorganisms (photosynthetic bacteria, lactic acid bacteria, yeasts, actinomycetes and fermenting fungi) has its own important function. However, photosynthetic bacteria are the pivot of activities of effective microorganisms. Photosynthetic bacteria support the activities of other microorganisms. On the other hand, photosynthetic bacteria also utilize substances produced by other microorganisms. This phenomenon is termed "coexistence and co-prosperity." Effective microorganisms are self-sterilizing (pH of between 3.4 and 3.7); therefore, majority of pathogens cannot survive in effective microorganisms medium (EMROSA, 2006). Photosynthetic bacteria are independent self-supporting microorganisms containing the

useful substances comprising of amino acids, nucleic acids, bioactive substances and sugars. These metabolites act as substrates for bacterial growth. Thus, increasing photosynthetic bacteria enhances other effective microorganisms (Higa, 1993). Lactic acid bacteria produce lactic acid from sugars, and other carbohydrates produced by photosynthetic bacteria and yeast. Thus, food and drinks, such as yogurt and pickles have been made by using lactic acid bacteria for a long period of time. However, lactic acid is a strong sterilizer. It suppresses harmful microorganisms and increases rapid decomposition of organic matter. Moreover, lactic acid bacteria enhance the breakdown of organic matter such as lignin and cellulose, and ferment these materials without causing harmful influences arising from un-decomposed organic matter (Higa & Parr, 1994). The yeasts that are present in effective microorganisms have a wide range of functions. They produce antimicrobial substances to kill harmful pathogens. In addition, they also produce beneficial substances, such as hormones, enzymes and vitamin B. Their secretions are useful substrates for other effective microorganisms such as lactic acid bacteria and actinomycetes (Higa & Parr, 1994). Yeasts synthesize antimicrobial and useful substances from amino acids and sugars secreted by photosynthetic bacteria, organic matter, etc. (Higa & Parr, 1994; Higa, 1993). Actinomycetes, the structure of which is intermediate to that of bacteria and fungi, produce antimicrobial substances from amino acids secreted by photosynthetic bacteria and organic matter. These antimicrobial substances suppress harmful fungi and bacteria. Actinomycetes can co-exist with photosynthetic bacteria. Fermenting fungi such as aspergillus and penicillium decompose organic matter rapidly to produce alcohol, esters and antimicrobial substances. These substances suppress odours and prevent infestation of harmful insects and maggots (Higa, 1993).

SCD Probiotics (2010) showed that the improvement in animals' performance after the use of effective microorganisms can be related to the inoculation of the gastro-intestinal tract with beneficial microorganisms. The gastro-intestinal tract of birds is host to approximately 40 species of microorganisms with three or more different types of each one. The flora plays an important role in the digestion process. Bacterial enzymes

promote the digestion of protein, lipids and carbohydrates, and bacteria also synthesize vitamins that contribute to the nutrition of the bird (Larbier & Leclercq, 1994). According to Yongzhen & Weijiong (1994) effective microorganisms improved the co-efficient of nitrogen absorption in the animals. Yongzhen & Weijong (1994) also found that the concentration of amino acids in the feed was improved by 28 % after the fermentation process with effective microorganisms, indicating that effective microorganisms improve the quality of the feed.

Application of effective microorganisms to both feed and drinking water reduces the ammonia concentration in the chicken house. Wei-jong & Yong-zhen (1994) found that effective microorganisms significantly decreased the incidence of contagious intestinal diseases such as bacillary white diarrhoea. Safalaoh (2006) suggested that effective microorganisms may regulate the composition of bacterial flora in the digestive tract and accelerate growth and development of chickens. Moreover, they prevent intestinal infections and speed up recoveries after illness. Wei-jong *et al*. (1994) showed that chickens treated with effective microorganisms increased resistance to diseases. Incong (1994) found that effective microorganisms added either in feeds or water did not cause any toxic effects on the chickens. It was further observed that the chickens did not develop any abnormalities or diseases in their skin and internal organs. Their respiration, feed and water uptake and growth rate were normal.

Mohan *et al*. (1996) suggested that the use of effective microorganisms has some beneficial effects in chicken production such as prevention of intestinal infections, and improved nitrogen utilization. It was further observed that effective microorganisms maintained intestinal microbial balance and helped gut mucosa development, improving digestion and absorption rate and, thus, improving production (Incong, 1994; Wondmeneh *et al*., 2011). Incong (1994) showed that improving the balance of the healthy microbes in digestive tract of broiler chickens can provide benefits ranging from stimulation of the immune system to reduction in the risk of certain illnesses. Wondmeneh *et al*. (2011) showed that additional benefits can be gained by

supplementing broiler diets with effective microorganisms. Oude- Elferink *et al*. (2001) showed that effective microorganisms aid the immunological response, which manifests in increased levels of antibodies, stimulation of the phagocytary activities of macrophages and increased number of cytotoxic cells. Ke *et al*. (2005) did not observe any significant changes in haematological and biochemical indexes examined. However, a slight decrease in serum cholesterol content (6.8 %) was reported by Safalaoh & Smith (2001) on the 42nd day of administering effective microorganisms to hens.

Hussain *et al.* (1994) found that nitrogen content of poultry manure increased after composting with effective microorganisms. Buihuu (2009) showed that supplementing broiler chickens with effective microorganisms increased protein content of the manure to 16.6 %. The contents of dry matter, total ash, calcium and fibre were considerably high. Some of the benefits claimed to accrue from the use of effective microorganisms include improved meat and manure quality, improved animal health, reduction of foul smells and absence of toxic effects on growing birds (Phillips & Phillips, 1996). However, others found no benefits at all (Ergun *et al*., 2000; Pelicano *et al*., 2004).

2.3 Influence of effective microorganism supplementation on productivity of chickens

Kumprechtova *et al*. (2000) indicated that the use of effective microorganisms as supplements improved feed intake, weight gain and feed conversion ratio in broiler chickens. Similarly, Botlhoko (2009) found that addition of effective microorganisms improved broiler chicken performance in terms of water intake, live weight and feed conversion ratio. Ashraf *et al*. (2005) reported that feed intake was higher in broiler chickens supplemented with effective microorganisms than the control group. Feed intake was significantly higher for broiler chickens supplemented with effective microorganisms in feed and water followed by those fed diets without effective microorganism supplementation during the starter phase. It was further observed that during the finisher phase, intake was significantly higher for the birds supplemented with

effective microorganisms than for those without being supplemented with effective microorganisms (Wondmeneh *et al*., 2011). Hussain (2000) also showed that addition of effective microorganisms to the diets or drinking water of broiler chickens improved body weight and quality of products. Mohan *et al*. (1996) suggested that the use of effective microorganisms has some beneficial effects in chicken production such as improvements in growth rate and feed efficiency. Yongzhen & Weijing (1994) observed that effective microorganisms improved the utilization of the feed and body weight gain of the chickens.

Deiver *et al*. (2008) showed that effective microorganisms significantly improved weight gain and feed conversion rate in male birds. However, female birds supplemented with effective microorganisms did not significantly respond to treatment with respect to weight gain and feed conversion ratios. SCD Probiotics (2010) found that effective microorganisms supplemented to the diets improved feed conversion ratio and daily live weight gain of broiler chickens. It was further observed by the author that after 45 days of effective microorganism supplementation live weight was higher in broiler chickens given effective microorganisms in both feed and drinking water followed by broiler chickens given effective microorganisms in drinking water and in feed then followed by those not supplemented with effective microorganisms. Similarly, Anjum (2011) showed that live weight was significantly higher in all groups treated with effective microorganisms compared with those not treated with effective microorganisms. Lokapirnasari (2007) showed that weight gain was significantly influenced by adding up to 4 cc of effective microorganisms per kg feed. However, Wondmeneh *et al*. (2011) found that addition of effective microorganisms had little effect on growth and feed conversion ratio of broiler chickens. Similarly, Ergun *et al*. (2000) found that supplementation of effective microorganisms to the diets had no effect on the performance of broiler chickens. Mohit *et al*. (2007), also, indicated that the use of effective microorganisms in broiler chickens did not result in significant increases in feed intake, weight gain and feed conversion ratio.

Safalaoh & Smith (2001) showed that chickens supplemented with effective microorganisms had significantly lower weight gains than those of the control groups. Safalaoh (2006) found that broiler chickens fed diets supplemented with effective microorganisms had lower feed intake than those not supplemented with effective microorganisms. Incong (1994) showed that effective microorganisms did not adversely affect the weight of the chickens. The authors, also, observed that there was no significant difference in the quality of the meat between the chicken supplemented with effective microorganisms and those not supplemented with effective microorganisms. Pandey (2001) showed that chickens supplemented with effective microorganisms had significantly lower live weight gain than the control group. Other studies (Sokol *et al*., 2009) also showed that administration of effective microorganisms to the diets of hens did not significantly influence their body weight and productivity.

Ashraf *et al*. (2005) found that broiler chickens given effective microorganisms had lower mortality rates than the control groups. Similarly, Vicente *et al*. (2007) reported that effective microorganisms significantly reduced mortality in broiler chickens. Wei-jong & Yong-zhen (1992) observed that the mortality of the birds given effective microorganisms decreased more than 35 percent compared with the control group. It was further observed by Wei-jong & Yong-zhen (1992) that mortality level of the effective microorganisms-treated birds during early growth (1 to 6 weeks), decreased by 80 percent compared with the control group. During the later growth stages, that is 7 to 20 weeks and 21 to 57 weeks, mortality level of effective microorganisms-treated birds decreased by 59 and 14 percent, respectively, compared with the control groups. Deiver *et al*. (2008) showed that supplementing effective microorganisms significantly reduced mortality in male broiler chickens. However, Wondmeneh *et al*. (2011) showed that effective microorganism supplementation had no significant effect on mortality of broiler chickens during the starter (1 to 29 days) and finisher (30 to 49 days) phases.

2.4 Influence of effective microorganism supplementation on carcass characteristics of chickens

Safalaoh (2006) found that effective microorganisms reduced abdominal fat pads and fat content of broiler chickens. Kalavathy *et al*. (2006) reported that addition of effective microorganisms to the diets of broiler chickens improved meat and carcass quality. Similarly, Safalaoh (2006) reported that dressing percentage was significantly higher for birds fed effective microorganisms. Safalaoh & Smith (2001), also, showed that effective microorganism supplementation significantly improved the dressing percentage of broiler chickens. However, Chantsavang & Watcharangkul (1999) observed that dietary effective microorganism supplementation had no effect on carcass characteristics of broiler chickens. The authors observed that dietary supplementation of effective microorganisms did not have an effect on crude protein percentage and monosaturated and polyunsaturated fatty acids of the broiler chicken meat. It was further observed that ash contents in breast meat were lower in chickens supplemented with effective microorganisms than in meat from the control group. Willis & Reid (2008) reported that significantly higher carcass yields were noted in males fed diets without effective microorganisms (78.1 %) than in those supplemented with effective microorganisms (74 %). However, female chickens supplemented with effective microorganisms had higher carcass yields than chickens not supplemented with effective microorganisms. Anjum *et al*. (2011) found that liver weight was significantly lower in birds supplemented with effective microorganisms than in those not supplemented with effective microorganisms. The authors, also, observed that gizzard weights did not differ significantly between effective microorganism-treated and non-treated broiler chickens. It was, also, observed that intestine length was significantly lower in effective microorganism- treated broiler chickens compared with the control group. Anjum *et al*. (1996) found that effective microorganism-treated birds had a decreased liver weight, gizzard weight and intestine length.

2.5 Conclusion

A number of studies have been done on supplementing diets with effective microorganisms in broiler chickens. These studies are extensive but not conclusive. There is also lack of information on supplementation levels for optimum productivity. Thus, there is need to do more studies on the influence of supplementing diets with effective microorganisms on feed intake, digestibility, live weight, growth, feed conversion ratio, mortality and carcass characteristics of Ross 308 broiler chickens. Such information will be valuable to farmers in South Africa and elsewhere with regard to improving the productivity of their chickens.

CHAPTER 3

MATERIALS AND METHODS

3.1 Study site

This study was conducted at the University of Limpopo Experimental Farm at Syferkuil, South Africa. The farm is located at about 10 km northwest of the Turfloop campus. The mean temperatures in winter (April to July) range between 10.1 and 28.4 °C and in summer (August to March) they range between 18 and 36 °C. The mean annual rainfall ranges from 446.8 to 468.4 mm (Shaker *et al*., 2009).

3.2 Preparation of effective microorganisms

Effective microorganisms (EM) were supplied by ZZ2 Company (Mooketsi, South Africa). The material contained a mixture of lactic acid bacteria with 8.3 x 10^6 CFU/mL (*Lactobacillus plantarium species*), yeasts with 1.8 x 10^5 CFU/mL (*Candida Valida Species*), actinomycetes with 3 x 10^3 CFU/mL (*Streptomyces albus species*) and fermenting fungi with 1.1 x 10^5CFU/mL (*Aspergillus Oryzae Species*). The EM solution was a yellowish liquid with a pleasant odour and sweet-sour taste. It was kept at a pH of 3 to enhance preservation. Effective microorganisms were added daily to the drinking water in the chicken fountains as specified in Section 3.3 below.

3.3 Experimental designs, treatments and procedures

The first part of this study commenced with 200 unsexed day-old Ross 308 broiler chicks and was carried out for a period of three weeks. Chicks from each replicate had an initial live weight of 50 ± 3 g per bird. The chickens were randomly allocated to five treatments (Table 3.01) with four replications, each having 10 chicks. A complete randomized design (SAS, 2008) was used. The area of each pen was 3 m^2. The diets were formulated to meet the nutrient requirements of the chickens as specified by McDonald *et al*. (2011). The diet ingredients are presented in Table 3.02. The diet contained 880 g DM/kg, 16.96 MJ of energy/kg DM, 220 g of crude protein/kg DM, 10 g of calcium/kg DM, 5.5 g of phosphorus/kg DM, 11.5 g of lysine/kg DM, 60 g of crude fibre/kg DM and 25 g of fat/kg DM.

Light was provided 23 hours daily while feed and water were provided *ad libitum* throughout the experiment. This part of the study was terminated when the chickens reached the age of 21 days.

Table 3.01 Diets for the chickens aged between one and 21 days

Diet code	Diet description
EM_0	Ross 308 broiler chickens fed a grower diet (22 % CP) without effective microorganism supplementation.
EM_{30}	Ross 308 broiler chickens fed a grower diet (22 % CP) supplemented with 30 ml of effective microorganisms per litre of water.
EM_{50}	Ross 308 broiler chickens fed a grower diet (22 % CP) supplemented with 50 ml of effective microorganisms per litre of water.
EM_{70}	Ross 308 broiler chickens fed a grower diet (22 % CP) supplemented with 70 ml of effective microorganisms per litre of water.
EM_{100}	Ross 308 broiler chickens fed a grower diet (22 % CP) supplemented with 100 ml of effective microorganisms per litre of water.

Table 3.02 Diet composition of grower mash for Ross 308 broiler chickens

Ingredient	g/kg feed
Yellow maize	567
Sunflower meal	100
Full fat soy meal	290
Fish meal	10
Monocalcium phosphate	13.6
Limestone	13.6
Iodised salt	0.5
DL Methionine	0.3
L Threonine	0.0
Vitamin/mineral premix	5.0
Total	1000
Nutrients	
Crude protein (%)	22
Energy (MJ/kg DM)	16

The second part of the study commenced with male Ross 308 broiler chickens aged 21 days. These were not the same chickens used in the first part of the study. The chickens had been raised on a grower mash (Table 3.02) until they were 21 days. Only male chickens were used because there were not enough females. A total of 60 male chickens aged 21 days, weighing 474 ± 2 g per chicken, were used for this part of the study. The experiment was terminated when the chickens were six weeks old. The

chickens were fed a grower diet. Thus, there were five treatments, replicated four times with four chickens per replicate, in a complete randomized design (SAS, 2008). The experimental treatments were as specified in Table 3.03 below. The diet ingredients used are the same as those used in the first experiment.

Table 3.03 Dietary treatments for the chickens aged 21 to 42 days

Diet code	Diet description
EMM_0	Male Ross 308 broiler chickens fed a grower diet (22 % CP) without effective microorganism supplementation.
EMM_{30}	Male Ross 308 broiler chickens fed a grower diet (22 % CP) supplemented with 30 ml of effective microorganisms per litre of water.
EMM_{50}	Male Ross 308 broiler chickens fed a grower diet (22 % CP) supplemented with 50 ml of effective microorganisms per litre of water.
EMM_{70}	Male Ross 308 broiler chickens fed a grower diet (22 % CP) supplemented with 70 ml of effective microorganisms per litre of water.
EMM_{100}	Male Ross 308 broiler chickens fed a grower diet (22 % CP) supplemented with 100 ml of effective microorganisms per litre of water.

3.4 Data collection

The initial live weights of the chickens were taken at the start of the experiment. Thereafter, weekly mean live weights and feed intakes were measured until termination of the experiment. Daily mean live weights and feed intakes were calculated from the weekly measurements. Daily growth rates and feed conversion ratios were also calculated. Mortality was recorded throughout the experiment. Digestibility was measured when the chickens were between 16 and 21 days old and between 36 and 42 days old. Digestibility was conducted in specially designed metabolic cages having separated watering and feeding troughs. Two birds were randomly selected from each

replicate and transferred to metabolic cages for the measurement of apparent digestibility. A three-day acclimatization period was allowed prior to a three-day collection period. Droppings voided by each bird were collected on a daily basis at 9.00 hours. Care was taken to avoid contamination from feathers, scales, debris and feeds. Apparent digestibility of the nutrients was calculated according to the procedures of McDonald *et al*. (2011) as follows:

Apparent digestibility (decimal) = (Amount of nutrient ingested – amount of nutrient excreted)/amount of nutrient ingested

At 42 days of age all remaining broiler chickens per pen were slaughtered by cervical dislocation as recommended by the University of Limpopo Ethics Committee. Carcass characteristics were then determined. Carcass parts and abdominal fat pads were weighed. Fat surrounding the gizzard and intestines, extending to the bursa, was considered as abdominal fat pad (Mendonca & Jensen, 1989).

Sensory evaluation involved testing for meat juiciness, flavour and tenderness (Xi, 2000). The meat samples were cooked to an internal temperature of 71 °C for 30 minutes. Samples were served hot after cooking. The following sensory attributes were evaluated by the sensory panel: tenderness, juiciness and flavour of meat samples. The sensory panel consisted of trained panellists. The scores used were as indicated in Table 3. 04.

Table 3.04 Evaluation scores used by the sensory panel

	Meat characteristics		
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

3.5 Chemical analysis

Dry matter of feeds, feed refusals, faeces and meat samples were determined by drying the samples in the oven for 24 hours at a temperature of 105 °C. Ash content of the feeds, faeces and meat were determined by ashing a sample at 600 °C in a muffle furnace overnight. Crude protein contents of the samples were determined by the Kjedahl method (AOAC, 2008). Gross energy values for feeds and faeces were measured in a bomb calorimeter (AOAC, 2008) at the University of Limpopo. Lysine, calcium, phosphorus, crude fibre and fat were determined (AOAC, 2008). Effective microorganisms were analysed (see section 3.2) by the supplier (ZZ2 Company, Mooketsi, South Africa) (AOAC, 2008).

3.6 Data analysis

Data on feed intake, digestibility, live weight, growth rate, feed conversion ratio, mortality and carcass characteristics of broiler chickens were analysed by general linear

model procedures for statistical analysis of variance (SAS, 2008). Duncan's Test for multiple comparisons was used to test the significance of differences between treatment means ($P<0.05$) (SAS, 2008).

CHAPTER 4

RESULTS

4.01 Influence of effective microorganisms on productivity of Ross 308 broiler chickens aged one to 21 days

Results of the influence of effective microorganism supplementation in the diets of Ross 308 broiler chickens on live weight, feed intake, growth rate and feed conversion ratio of broiler chickens aged between one and 21 days are presented in Table 4.01. Effective microorganism supplementation had no influence ($P>0.05$) on feed intake of the chickens. However, effective microorganism supplementation had influence ($P<0.05$) on growth rate, live weight and feed conversion ratio of the chickens. Chickens supplemented with 50, 70 or 100 ml of effective microorganisms per litre of water had poorer ($P<0.05$) feed conversion ratios than those not supplemented with effective microorganisms. Similarly, chickens supplemented with 50, 70 or 100 ml of effective microorganisms per litre of water had lower ($P<0.05$) live weights and growth rates than those not supplemented with effective microorganisms. Broiler chickens not supplemented with effective microorganisms and those supplemented with 30 ml of effective microorganisms per litre of water had similar ($P>0.05$) growth rates, live weights and feed conversion ratios. Broiler chickens supplemented with 30, 50, 70 or 100 ml of effective microorganisms per litre of water had similar ($P>0.05$) growth rates, live weights and feed conversion ratios. Effective microorganism supplementation to the diets of broiler chickens reduced ($P<0.05$) mortality rate from 10 % to 0 %.

Results of the influence of effective microorganism supplementation to the diets of Ross 308 broiler chickens on dry matter digestibility, apparent metabolisable energy and crude protein retention of the chickens aged three weeks are presented in Table 4.02. Chickens not supplemented with effective microorganisms and those supplemented with 30 ml of effective microorganisms per litre of water had lower ($P<0.05$) crude protein retention values than those supplemented with 50, 70 or 100 ml of effective microorganisms per litre of water. Broiler chickens supplemented with 50, 70 or 100 ml of effective microorganisms per litre of water had same ($P>0.05$) crude protein retention values. Chickens not supplemented with effective microorganisms and those

supplemented with 30 ml of effective microorganisms per litre of water had similar (P>0.05) crude protein retention values. Broiler chickens supplemented with 100 ml of effective microorganisms per litre of water had higher (P<0.05) diet DM digestibility values than those supplemented with 30 ml of effective microorganisms per litre of water. Broiler chickens not supplemented with effective microorganisms and those supplemented with 30, 50 or 70 ml of effective microorganisms per litre of water had similar (P>0.05) dry matter digestibility values.

Table 4.01 Effect of supplementing diets with effective microorganisms on live weight at 21 days of age (g/bird), feed intake (g/bird/day), growth rate (g/bird/day) and feed conversion ratio (FCR) (g DM feed/g live weight gain) of unsexed broiler chickens aged one to 21 days

	Variable			
Diet code	Live weight	Intake	Growth rate	FCR
EM_0	512.8^{a}	50.8	24.4^{a}	2.1^{b}
EM_{30}	482.3^{ab}	51.2	22.9^{ab}	2.2^{ab}
EM_{50}	464.7^{b}	52.2	22.1^{b}	2.4^{a}
EM_{70}	460.1^{b}	51.3	22.0^{b}	2.3^{a}
EM_{100}	452.3^{b}	51.8	21.5^{b}	2.4^{a}
SE	8.545	0.639	0.407	0.049

$^{a, b}$: Means in the same column not sharing a common superscript are significantly different (P<0.05).

SE : Standard error

Similarly, chickens not supplemented with effective microorganisms and those supplemented with 50, 70 or 100 ml of effective microorganisms per litre of water had the same (P>0.05) DM digestibility values. Chickens not supplemented with effective microorganisms had higher (P<0.05) ME intakes than those supplemented with 100 ml of effective microorganisms per litre of water. Broiler chickens not supplemented with effective microorganisms and those supplemented with 30, 50, or 70 ml of effective microorganisms per litre of water had similar (P>0.05) ME intakes. Similarly, chickens supplemented with 30, 50 or 70 ml of effective microorganisms per litre of water had the same (P>0.05) ME intakes.

Table 4.02 Effect of supplementing diets with effective microorganisms on diet dry matter (DM) digestibility (decimal), metabolisable energy (ME) (MJ/kg DM) and crude protein (CP) retention (g/bird/day) of unsexed broiler chickens aged 3 weeks

	Variable		
Diet code	DM digestibility	ME	CP retention
EM_0	0.64^{ab}	11.6^{a}	9.0^{b}
EM_{30}	0.60^{b}	11.3^{ab}	9.7^{b}
EM_{50}	0.64^{ab}	11.1^{ab}	11.3^{a}
EM_{70}	0.62^{ab}	11.0^{ab}	10.8^{a}
EM_{100}	0.65^{a}	10.9^{b}	10.9^{a}
SE	0.006	0.086	0.252

$^{a, b}$: Means in the same column not sharing a common superscript are significantly different (P<0.05).

SE : Standard error

4.02 Influence of effective microorganisms on productivity of Ross 308 broiler chickens aged 22 to 42 days

Results of the influence of effective microorganism supplementation in the diets of male Ross 308 broiler chickens on live weight, feed intake, growth rate and feed conversion ratio of Ross 308 broiler chickens aged three to six weeks are presented in Table 4.03. Effective microorganism supplementation had no influence ($P>0.05$) on intake and feed conversion ratio of male broiler chickens. However, effective microorganism supplementation had influence ($P<0.05$) on growth rate and live weight of male broiler chickens. Male broiler chickens not supplemented with effective microorganisms had higher ($P<0.05$) growth rates and live weights than those supplemented with effective microorganisms. Male chickens supplemented with effective microorganisms had similar ($P>0.05$) live weights and growth rates. Effective microorganism supplementation in the diets of male broiler chickens reduced ($P<0.05$) mortality rate from 5 % to 0 %.

Results of the influence of supplementing diets of male Ross broiler chickens with effective microorganisms on diet digestibility, metabolisable energy and crude protein retention of the chickens aged six weeks are presented in Table 4.04. Effective microorganism supplementation had no effect ($P>0.05$) on diet DM digestibility. However, effective microorganism supplementation had influence ($P<0.05$) on metabolisable energy and crude protein retention. Broiler chickens not supplemented with effective microorganisms and those supplemented with 50, 70 or 100 ml of effective microorganisms per litre of water had higher ($P<0.05$) metabolisable energy values than those supplemented with 30 ml of effective microorganisms per litre of water. Chickens not supplemented with effective microorganisms and those supplemented with 50, 70 or 100 ml of effective microorganisms per litre of water had similar ($P>0.05$) metabolisable energy values. Chickens supplemented with 100 ml of effective microorganisms per litre of water had higher ($P<0.05$) crude protein retention values than those not supplemented with effective microorganisms and those

supplemented with 30, 50 or 70 ml of effective microorganisms per litre of water. However, chickens not supplemented with effective microorganisms and those supplemented with 30, 50 or 70 ml of effective microorganisms had similar (P>0.05) crude protein retention.

Effective microorganism supplementation had no effect (P<0.05) on dressing percentage, fat pad, gizzard, wing, drumstick and liver weights, and intestinal length of male broiler chickens (Table 4.05). However, effective microorganism supplementation had influence (P<0.05) on carcass, thigh and breast meat of male broiler chickens (Table 4.05). Male chickens supplemented with 50, 70 or 100 ml of effective microorganisms per litre of water had lower (P<0.05) carcass and breast meat weights than those not supplemented with effective microorganisms. However, broiler chickens supplemented with effective microorganisms had similar (P>0.05) breast and carcass weights. Similarly, male broiler chickens not supplemented with effective microorganisms and those supplemented with 30 ml of effective microorganisms per litre of water had similar (P>0.05) breast and carcass weights. Male chickens not supplemented with effective microorganisms had higher (P<0.05) thigh weights than those supplemented with 30 or 100 ml of effective microorganisms per litre of water. Male chickens supplemented with 30, 50, 70 or 100 ml of effective microorganisms per litre of water had similar (P>0.05) thigh weights. Similarly, broiler chickens not supplemented with effective microorganisms and those supplemented with 50 or 70 ml of effective microorganisms per litre of water had similar (P>0.05) thigh weights.

Male Ross 308 broiler chickens supplemented with 100 ml of effective microorganisms per litre of water had higher (P<0.05) crude protein contents in their breast meat than those of the chickens not supplemented with effective microorganisms or supplemented with 30, 50 or 70 ml of effective microorganisms per litre of water. Similarly, male chickens not supplemented with effective microorganisms had higher (P<0.05) crude

protein contents in their breast meat than those supplemented with 30, 50 or 70 ml of effective microorganisms per litre of water.

Table 4.03 Effect of supplementing diets with effective microorganisms on live weight (g/bird aged 42 days), feed intake (g/bird/day), growth rate (g/bird/day) and feed conversion ratio (g DM feed/kg live weight gain) of male broiler chickens aged 21 to 42 days

	Variable			
Diet code	Live weight	Intake	Growth rate	FCR
EMM_0	1620[a]	155	77.1[a]	2.0
EMM_{30}	1278[b]	145	60.8[b]	2.4
EMM_{50}	1363[b]	133	64.9[b]	2.1
EMM_{70}	1365[b]	152	65.0[b]	2.3
EMM_{100}	1249[b]	143	59.5[b]	2.4
SE	34.513	3.706	1.643	0.066

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

SE : Standard error

Table 4.04 Effect of supplementing diets with effective microorganisms on diet dry matter (DM) digestibility (decimal), metabolisable energy (ME) (MJ/kg DM) and crude protein (CP) retention (g/bird/day) of male broiler chickens aged six weeks

	Variable		
Diet code	DM digestibility	ME	CP retention
EMM_0	0.62	11.0^a	9.2^b
EMM_{30}	0.65	10.3^b	9.6^b
EMM_{50}	0.65	10.8^a	10.9^b
EMM_{70}	0.66	11.2^a	9.74^b
EMM_{100}	0.66	10.9^a	13.0^a
SE	0.010	0.090	0.413

$^{a, b}$: Means in the same column not sharing a common superscript are significantly different ($P<0.05$).

SE : Standard error

Table 4.05 Effect of supplementing diets with effective microorganisms on carcass characteristics (g) of male Ross 308 broiler chickens aged 42 days

	Variable								
Diet Code	Dressing % (%)	Carcass	Intestine Length (cm)	Fat pad	Gizzard	D/stick	Thigh	Liver	Breast
EMM_0	88	2005[a]	233	34	40	208	235[a]	50	485[a]
EMM_{30}	88	1780[ab]	244	25	43	190	200[b]	43	430[ab]
EMM_{50}	84	1680[b]	221	30	40	197	202[ab]	40	378[b]
EMM_{70}	86	1728[b]	240	28	40	180	215[ab]	43	370[b]
EMM_{100}	86	1723[b]	201	28	45	182	190[b]	40	383[b]
SE	0.65	41.47	7.23	2.57	1.31	4.37	5.35	1.79	15.79

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

SE : Standard error

Table 4.06 Effect of supplementing diets with effective microorganisms on crude protein content (%) of breast meat of male Ross 308 broiler chickens aged 42 days

Diet code	Crude protein content
EMM_0	27.27^b
EMM_{30}	26.16^e
EMM_{50}	26.94^d
EMM_{70}	27.16^c
EMM_{100}	38.91^a
SE	1.1073

$^{a, b, c, d, e}$: Means in the same column not sharing a common superscript are significantly different (P<0.05).

SE : Standard error

Effective microorganism supplementation did not improve (P>0.05) meat flavour, tenderness and juiciness of male Ross 308 broiler chickens aged 42 days (Table 4.07).

Table 4.07 Effect of supplementing diets with effective microorganisms on flavour, tenderness and juiciness of meat of male Ross 308 broiler chickens aged 42 days

	Variable		
Diet	Flavour	Tenderness	Juiciness
EMM_0	2.36	2.20	2.30
EMM_{30}	2.28	2.37	2.37
EMM_{50}	2.50	2.09	2.40
EMM_{70}	2.55	2.42	2.47
EMM_{100}	2.42	2.17	2.30
SE	0.056	0.060	0.080

SE : Standard error

CHAPTER 5

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

Effective microorganism supplementation to the diets of Ross 308 broiler chickens aged one to three weeks, and male chickens aged three to six weeks had no effect on feed intake. This finding is similar to that reported by Ergun *et al*. (2000) who observed that supplementation with effective microorganisms to the diets had no effect on intake. Contrary to these results, Ashraf *et al*. (2005) found that feed intake was higher in broiler chickens supplemented with effective microorganisms than in those not supplemented with effective microorganisms. Similarly, Wondmeneh *et al*. (2011) observed that feed intake at both starter and finisher phase was significantly higher in broiler chickens supplemented with effective microorganisms than in those not supplemented with effective microorganisms. SCD Probiotics (2010) reported that improvement in intake after supplementation with effective microorganisms can be related to the inoculation of the gastro-intestinal tract with beneficial microorganisms. The gastro-intestinal tract of chickens is host to approximately 40 species of microorganisms with three or more different types of each one. The flora plays an important role in the digestion process. Bacterial enzymes promote the digestion of proteins, lipids and carbohydrates. Bacteria, also, synthesize vitamins that contribute to the nutrition of the chicken (Larbier & Leclercq, 1994). However, Safalaoh (2006) found that at finisher stage feed intake was lower in broiler chickens supplemented with effective microorganisms than the control group.

Results of the present study indicate that effective microorganism supplementation reduced growth rate of Ross 308 broiler chickens at both starter and finisher phases. This was, possibly, due to poorer feed conversion ratio with effective microorganism supplementation at the starter phase. This finding is similar to that obtained by Wondmeneh *et al*. (2011) who reported that addition of effective microorganisms to the diets reduced growth rate of chickens. Contrary to these, Yongzhen & Weijing (1994) observed that effective microorganism supplementation improved growth rate of broiler chickens. Similarly, Mohan *et al*. (1996) reported that effective microorganism supplementation to the diets improved growth rate of the chickens. Lokapirnasari

(2007), also, demonstrated that weight gain in broiler chickens was significantly improved with addition of up to 4 cc of effective microorganisms per kg feed. Safalaoh (2006) suggested that improvement of growth in chickens after effective microorganism supplementation may be due to the fact that effective microorganisms improve the composition of beneficial microbes in the digestive tract and hence there is an acceleration of digestion, intake and growth of chickens.

Supplementing effective microorganisms to the diets reduced live weights of the unsexed Ross 308 broiler chickens aged one to three weeks as well as those of male chickens aged three to six weeks. This may be due to lower growth rates and higher feed conversion ratios observed in chickens supplemented with effective microorganisms. Mohit *et al*. (2007), also, indicated that the use of effective microorganisms in broiler chickens did not result in significant increases in live weight gain. Contrary to these results, Kumprechtova *et al*. (2000) reported that effective microorganisms supplementation to the diets improved live weight gains in broiler chickens. Similarly, Anjum *et al*. (2011) observed that live weight was significantly higher in broiler chickens treated with effective microorganisms compared with those not treated with effective microorganisms. It was further observed by the authors that after 45 days of effective microorganism supplementation, live weight was higher in broiler chickens given effective microorganisms in both feed and drinking water followed by broiler chickens given effective microorganisms in drinking water or in feed than in chickens not supplemented with effective microorganisms. Deiver et al. (2008), also, reported that effective microorganisms significantly improved live weight gains in male broiler chickens.

Effective microorganism supplementation resulted in poorer feed conversion ratio of unsexed Ross 308 broiler chickens aged one to 21 days. Wondmeneh *et al*. (2011), also, found that addition of effective microorganisms resulted in poorer feed conversion ratio of broiler chickens. Contrary to these results, Botlhoko (2009) and Deiver *et al*. (2008) found that addition of effective microorganisms improved feed conversion ratio.

Similarly, Mohan *et al*. (1996) reported that the use of effective microorganisms as supplements has some beneficial effects on feed efficiency of the chickens. In the present study, effective microorganism supplementation had no influence on feed conversion ratio of chickens aged three to six weeks. Similar results were reported by Mohit *et al*. (2007).

Supplementing diets of broiler chickens with effective microorganisms reduced mortality rate from 10 % to 0 % (starter stage) and 5 % to 0 % (finisher stage). Wei-jong *et al*. (1994) found similar results. These authors found that effective microorganisms significantly decreased the incidence of contagious intestinal diseases such as bacillary white diarrhoea. Thus, effective microorganism supplementation prevented intestinal infections and hence quickened recoveries after illness (Wei-jong *et al*., 1994; Yong-zhen & Wei-jong, 1994). Wei-jong *et al*. (1994) showed that chickens treated with effective microorganisms increased resistance to diseases, thus reducing mortality of the chickens. This finding is similar to that obtained by Ashraf *et al*. (2005). Similarly, Vicente *et al*. (2007) and Deiver (2008) found that effective microorganism supplementation significantly reduced mortality in chickens. Yong-zhen & Wei-jong (1994) showed that mortality of the chickens given effective microorganisms decreased more than 80 %. However, Wondmeneh *et al*. (2011) found that effective microorganism supplementation had no significant effect on mortality of chickens during the starter and finisher phases.

Effective microorganism supplementation to the diets did not improve dressing percentage, carcass, fat pad and carcass part weights of Ross 308 broiler chickens. Chantsavang & Watcharangkul (1999) and Anjum *et al*. (2011) found similar results in broiler chickens. However, Safalaoh (2006) found that effective microorganism supplementation to the diets reduced fat pads and fat contents of broiler chickens. Similarly, Willis & Reid (2008) found that chickens supplemented with effective microorganisms had lower carcass weight than those not supplemented with effective microorganisms. However, Safalaoh & Smith (2001) reported that effective

microorganism supplementation significantly improved the dressing percentage of broiler chickens.

Supplementing male Ross 308 broiler chickens with effective microorganisms improved crude protein retention. This resulted in improved crude protein content of the meat from chickens supplemented with effective microorganisms. No information on crude protein contents of meat from chickens supplemented with effective microorganisms was found. Supplementing diets of the chickens with effective microorganisms did not improve dietary dry matter digestibility and metabolisable energy. These results are contrary to those of Buihuu (2009) who showed that supplementing diets of broiler chickens with effective microorganisms improved dietary dry matter digestibility and metabolisable energy.

Supplementing diets of male Ross 308 broiler chickens with effective microorganisms did not improve their meat tenderness, flavour and juiciness. Incong (1994) found that effective microorganism supplementation to the diets of broiler chickens improved flavour and juiciness of their meat. Hussain (2000) also found that addition of effective microorganisms to the diets or drinking water of broiler chickens improved quality of their meat.

5.2 Conclusion

It is concluded that effective microorganism supplementation to the diets of Ross 308 broiler chickens reduced mortality rate of the chickens. This finding is very promising for rural chicken farms where disease prevalence is very high. It can, also, be concluded that effective microorganism supplementation improved crude protein retention and crude protein content of the meat of male Ross 308 broiler chickens aged six weeks. However, this improvement did not have any effect on meat tenderness, flavour and juiciness. Supplementation of diets with effective microorganisms reduced growth rates

and live weights of Ross 308 broiler chickens. Similarly, effective microorganism supplementation did not improve intake of Ross 308 broiler chickens.

5.3 Recommendations

Biological reasons for a decrease in mortality when chickens are supplemented with effective microorganisms are not clear. It is, therefore, important to explore such reasons. More research is required to explore biochemical reasons for increase in crude protein retention and crude protein content of meat from broiler chickens supplemented with effective microorganisms.

CHAPTER 6

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CHAPTER 7

APPENDIX A

APPENDIX A: VACCINATION PROGRAMME

The vaccination programmes of the study were as indicated below:

Day one:	On arrival chicks were vaccinated against Newcastle disease from the hatchery using Clone 30.secondly, Vita stress was added in the drinking water immediately on arrival for the first two days to calm down the chicks due to stress they might have experienced through transportation and handling.
Day three:	Tylo tad was added in the drinking water for prevention of *Escheria coli* bacteria and other disease causing microorganisms.
Day seven:	Chicks vaccinated against Infectious bronchitis using “IBH 120”.
Day twelve:	Chicks were vaccinated against Gumbora using D78 through drinking water.
Day eighteen:	Chicks were vaccinated against Gumbora using D78 through drinking water.
Day twenty one:	Tylo tad was added in the drinking water.
Day twenty three:	Chickens were vaccinated against Newcastle disease using Clone.