ASSESSMENT OF EFFECTS OF EFFECTIVE MICROORGANISMS ON BROILER CHICKEN PERFORMANCE AND MALODOUR REDUCTION IN POULTRY HOUSE

BY

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ABSTRACT

The study was conducted to assess the effects of Effective Microorganisms (EM) as feed additive in broiler chicken production on growth performance, health and foul smell control in poultry house. The experiment involved two hundred and ten, day old hybro broiler chicks which were randomly allocated to 14 pens each with 15 birds in seven treatments with two replicates. The main treatment (EM) was provided to experimental birds in two levels: 10mlEM2/l and 20mlEM2/l either in drinking water or by spraying in litter material or both in water and litter. After 42 days of the experiment 70 live chickens were selected and 5 birds/pen from each replicate were slaughtered for carcass assessment.

There were significant difference between treatments on growth performance and carcass yield. EM treated groups had significant higher body weight gain, cumulative body weight gain and average daily body weight gain compared to no-EM control group. Carcass yield percent were comparatively higher in EM treated birds than control groups. T7 had significantly high carcass yield percent (79.13%) compared to other treatments and control group (73.91%). Internal organs such as gizzard and liver had significantly higher weights for EM treated birds compared with no-EM control birds. The overall mortality rate of birds was 12.8% and the highest mortality rate occurred in the first week (9%) and in the second week (3.3%), due to sudden death syndrome, salmonellosis, and huddling.

Significant differences were also observed between treatment effects and treatment combinations on ammonia concentration. Pens with birds receiving 20mlEM/l both in water and litter had significant low ammonia levels compared with control group. It is concluded that EM had growth promoting effects, reduced mortality rates and reduced significantly the ammonia levels in poultry house in higher dose of 20mlsEM2/l concurrently supplemented in water and sprayed in litter.

DECLARATION

I, HONORIUS DISMAS MGUNDA do hereby declare to the senate of Sokoine University of Agriculture that, this dissertation is my own original work and has neither been submitted nor concurrently been submitted for degree award in any other Institution.

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The above declaration is confirmed by,

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Dr. Mbaga, S. H **Date**

Supervisor

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DEDICATION

To my wife Dynes, my daughter Angelica, my sons Dismas, Emmanuel and Edward for their patience and encouragement.

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LIST OF ABBREVIATIONS

%	Percent
ADWTG	Average daily weight gain
ANOVA	Analysis of variance
BWT	Body weight
CONC.	Concentration
CUMWTG	Cumulative weight gain
CWT	Carcass weight
DASP	Department of Animal Science and Production
DWT	Dressed weight
EM	Effective microorganisms
EMRO	Effective Microorganisms Research Organization
FNWT	Final weight
FOS	Fructo-oligosaccharides
GIT	Gastrointestinal tract
GIWT	Gizzard weight
H_2S	Hydrogen Sulfide
HCl	Hydrochloric acid
i.e.	That is
INWT	Initial weight
l	Litre
LIWT	Liver weight
Lsmeans	Least square means
mg	Milligram/s
MOS	Mannan-oligosaccharides
N	Normality

NaOH	Sodium Hydroxide
NH_3	Ammonia
pH	Hydrogen ion concentration
ppm	Parts per million
REP	Replication
SAS	Statistical Analysis System
SCD	Sustainable Community Development
SUA	Sokoine University of Agriculture
T1	Treatment 1
T2	Treatment 2
T3	Treatment 3
T4	Treatment 4
T5	Treatment 5
T6	Treatment 6
T7	Treatment 7
TRT	Treatment
V	Volume
WK	Week

CHAPTER ONE

1.0 INTRODUCTION

Probiotics or microbial preparations have been increasingly used in livestock productions as feed supplement. Available literature suggest that use of microbial preparations e.g. Effective Microorganisms (EM), have some beneficial effects in poultry production such as improvements in growth rate, feed efficiency, prevention of intestinal infections and improved nitrogen utilization (Safalaoh, 2006). Probiotics are live microorganisms which when administered in adequate amounts, confer a beneficial health effects on the host (Zonis, 2008). EM is a combination of useful regenerative micro-organisms that exist freely in nature and are not manipulated in any way. EM Preparations are mainly made from Lactic acid bacteria, Yeasts, Actinomycetes, Photosynthetic bacteria and Fungi (EMRO, 2010).

Consumers' demand for natural and organic foods has risen steadily. Correspondingly, the demand for technologies to enhance animal performance through natural and organic solutions continues to increase. One such technology receiving considerable attention is the feedstuff application of live beneficial bacteria commonly called probiotics (Flint and Garner, 2009). Effective microorganisms are probiotics and were developed by Professor Teruo Higa of the University of Ryukyus, Okinawa, Japan in 1980s (Higa, 1994).

EM is widely used in livestock farming, because it is totally natural and effective method for ensuring healthy poultry and is ideal for use on both commercial and organic farmed units as well as domestic flocks. When EM is used in rearing sheds, not only helps to suppress disease causing microorganisms, but rapidly eliminate and control the ammonia produced from droppings and as a result the air quality is improved significantly. EM when added to the feed and water , the beneficial microorganisms tend to improve the gut

flora of the birds, making digestion more efficient and thereby helping to reduce feed costs. When EM is added in bird's diet, it increases growth rate, egg production and reproductive performance (EMRO, 2010).

In crop production, Effective Microorganisms help significantly to increase soil fertility and increase plants' growth and resistance. The Effective Microorganisms promote regenerative microorganisms and organisms in the soil. They help to convert organic materials into nutrients that are available for plants and create an environment in which the pathogenic bacteria and pests are removed from their habitat (Multikraft, 2010). EM is effective in malodour control in environment. In a study on the production of broiler chickens it was observed that concentration of toxic Ammonia (NH_3) inside a poultry house accommodating EM treated birds was 26.5 ppm compared with 87.6 ppm for non-EM control groups. EM can also effectively transform NH_3 into less toxic substances therefore maintaining a safer environment to birds and attendants as well (Li, *et al.*, 1998).

Effective microorganisms are a developing technology that may support the profitability and safety of poultry production and offer meaningful alternatives for natural and organic production. The main objective of this study was to assess the effects of Effective Microorganisms (EM) as feed additive in broiler chickens production on body weight, health and foul smell control in poultry house.

The specific objectives were:

i. To assess performance of broiler chickens raised on basal diets supplemented with water-based Effective Microorganisms,
ii. To assess general health and mortality on broiler fed basal diet supplemented with water-based Effective Microorganisms,

iii. To assess the efficacy of Effective Microorganisms for controlling foul smell in poultry house.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Growth Promoters

Growth promoters are chemical and biological substances which are added to livestock food with the aim to improve the growth of chickens in fattening, to improve utilization of food and in this way realize better production and financial returns. Their mechanism of action varies. Positive effects can be expressed through better appetite, improved feed conversion, stimulation of the immune system, increased vitality and regulation of the intestinal micro-flora. In any case, expected results of the use of these additives are increased financial returns on production (Peric, *et al*., 2009). Different groups of growth promoters have different mechanisms of action and therefore, they present various effects upon their utilization. The most popular growth promoters are Antibiotics, probiotics, prebiotics, synbiotics, enzymes, acidifiers, antioxidants and phytobiotics (Peric, *et al*., 2009).

2.2 Antibiotic Growth-promoters

The term "antibiotic growth promoter" is used to describe any medicine that destroys or inhibits bacteria and is administered at a low sub-therapeutic dose. The use of antibiotics for growth promotion has arisen with the intensification of livestock farming. Infectious agents reduce the yield of farmed food animals and to control these, the administration of sub-therapeutic antibiotics and antimicrobial agents has been shown to be effective. Currently, there is controversy surrounding the use of growth promoters for animals intended for meat production, because overuse of any antibiotic over a period of time may lead to the local bacterial populations becoming resistant to the antibiotic. Human health can either be affected directly through residues of an antibiotic in meat, which may cause side-effects, or indirectly, through the selection of antibiotic resistance determinants that

may spread to human. For example, a drug that demonstrates both potential problems is chloramphenicol (Hughes and Heritage, 2010).

The practice of feeding livestock with sub-therapeutic levels of antibiotics (i.e. used for animal growth promotion rather than specifically to treat disease symptoms) has been in use for over fifty years (Markovic, *et al*., 2009). Antibiotics affect microflora by altering the metabolism of microorganisms and suppressing microbial growth in the gut. Usage of antibiotics has negative effects on animal's health such as residual in tissues, long withdrawal period development of resistance in microorganisms, allergies and genotoxicity (Markovic, *et al*., 2009). As the demand of consumers for organic and naturally produced livestock increases, the use of antibiotics in animal feed will decrease and will be limited to therapeutic treatment of diseases or will be eliminated outright in evolving animal production systems (Flint and Garner, 2009).

However, to date, antibiotic use has become an essential tool to increase animal productivity in concentrated animal-feeding and animal-rearing operations. Remarkable increases in improved profitability are seen with the adoption of antibiotic application at sub-therapeutic levels. It is estimated that 11.2 million kg of antibiotics are used annually as growth promoters in livestock (Flint and Garner, 2009). Consequently, it is hypothesized that widespread use of antimicrobial agents has led to increases in antimicrobial-resistant pathogens. As an example, in 2001, 19% (75/384) of human *Campylobacter* isolates were resistant to ciprofloxacin, whereas from 1989 to 1990 no ciprofloxacin-resistant strains were identified (Flint and Garner, 2009).

2.3 Probiotics

Over the past few years, a great deal of interest has been generated on the evaluation of alternative means for manipulation of gastrointestinal microflora in livestock. The motivation for examining these alternatives comes from increased public concern about the use of antibiotics in the animal feed industry as well as the need for a safe food supply (Markovic, *et al*., 2009). Probiotics are one of the alternative growth promoters, which in most cases have demonstrated positive effects on health and performance of broiler chickens. This shows that application of alternative growth promoters in nutrition of fattening chickens would be more efficient (Peric, *et al.*, 2009).

A number of Probiotics are available in the market with different trade and commercial names bearing very high publicity. Probiotic is a combination of beneficial bacteria adaptable to the intestinal mucosa of all warm blooded animals. However, the primary bacterial organism is *Lactobacillus acidophilus*, which implants itself on the villi of the intestinal wall in astronomical numbers thereby creating an acid environment through the very nature of the organism which grows best at pH of 5.0 to 6.5. The implantation through an effect called crowding begins to take over the environment of the gut producing eventually an excellent state of intestinal health, creating an appetite and making available medium for the complete digestion, absorption and assimilation of all nutrients being acted upon by the intestine (Tariq, *et al.*, 2005).

2.3.1 Properties of Probiotics

Characteristics of the ideal probiotics are to be of host origin, non-pathogenic, withstand processing and storage, resist gastric acid and bile, adhere to epithelium or mucus, persist in the intestinal tract, produce inhibitory compounds, modulate immune response and alter microbial activities (Patterson and Burkholder, 2003).

2.3.2 Mode of Action of Probiotics

The mode of action by which probiotics elicit their beneficial effects on the host tend to vary widely. Some effects result from direct interactions with the host epithelia and immune system, while others mediate their effects via modulation of resident intestinal flora and prevention of pathogen establishment. Conveniently, the modes of action of probiotics can be classified into 3 main categories: chemical inhibition, competitive exclusion, and microbially mediated immunodevelopment (Flint and Garner, 2009). The mode of action by competitive exclusion means that there is competition for attachment sites in the gastrointestinal tract (GIT). The bacteria of the probiotic attach to the intestinal mucosa, thereby forming a physical barrier that blocks the attachment of pathogenic bacteria .They also produce antibacterial compounds and enzymes (Dunkley, 2008).

2.4 Prebiotics

Prebiotics are defined as non-digestible food components or ingredients which have positive effect on host in their selective growth and/or activation of certain number of bacterial strains present in intestines. The most significant compounds which belong to group of prebiotics are oligosaccharides: fructo-oligosaccharides (FOS), gluco-oligosaccharides and mannan oligosaccharides (MOS). Their advantage compared to probiotics is that they promote growth of useful bacteria which are already present in the host organism and are adapted to all conditions of the environment. Favourable effects of addition of probiotics reflect in presence of antagonism towards pathogens, competition with pathogens, promotion of enzyme reaction, reduction of ammonia, phenol production and increase of resistance to colonization. Similar to probiotics, results of the effects on broiler performance are contradictory. In analysing the effects of implementation of FOS on broiler performances it was established that improvement of gain was 5-8% and improvement of feed conversion was 2-6% (Peric, *et al.*, 2009).

Several studies have shown that addition of prebiotics to the diet of broiler, layer and pig leads to improved performance through improving gut microflora (Markovic, *et al*., 2009). In contrast to the antibiotic mode of action, which limits or suppresses growth of common Gram-positive microflora, mannan-oligosaccharides and other oligosaccharides can prevent attachment of Gram-negative pathogens to enterocytes. The benefits of MOS are based on different specific properties including reduction of the intestinal mucous cells turnover rate. These properties have the potential to enhance growth rate and feed conversion in commercial broilers diet. Bacterial pathogens which bind to the intestinal wall via mannose bearing lectins, bind to the dietary MOS instead and are eliminated harmlessly (Markovic, *et al*., 2009).

Hence basically the mechanism in which prebiotics act is by supplying nutrients to beneficial microbes or tricking pathogenic bacteria into attaching to the oligosaccharide rather than to the intestinal mucosa. This reduces the intestinal colonization thereby decreasing the incidence of infection in the birds. Because the oligosaccharide is non-digestible, the microbes that are attached will travel along the GIT with the ingesta and are eventually excreted from the bird along with other undigested food (Dunkley, 2008).

2.5 Synbiotics

This is relatively a recent term among additives used in poultry nutrition. Synbiotics combination is primarily of probiotics and prebiotics, as well as other promoting substances which together exhibit joint effects in regard to health of digestive tract, digestibility and performances of broilers. Investigations showed that combinations used in synbiotics are often more efficient in relation to individual additives (Peric, *et al.*, 2009).

2.6 Effect of Probiotics on Poultry Performance

The study by Shareef and Al-Dabbagh (2009) have shown that adding yeast (*Saccharomyces cerevisiae*) in feed for three weeks at the rate of 1, 1.5 and 2% in all treated broiler chicks, have significant increase on body weight gain, feed consumption and feed conversion. Flint and Garner (2009) reported that measurements of body weight gain in poultry receiving probiotics treatment have shown variable results, also noted a dose-dependent response with probiotics application. The authors further noted reduced mortality in chickens receiving 0.10% (wt/wt) *Lactobacillus* culture from 8.2% in control birds to 3.2% in EM treated birds. These animals also demonstrated superior food conversion ratio. However, higher doses (0.15% wt/wt) resulted in lower productivity near that of the control group receiving no probiotics. Similar results using EM at the rate of one part EM to 1000 parts water, have been revealed by Safalaoh (2006) who suggested that microbial preparations such as EM, can be used to improve weight gain, feed utilisation and reduce abdominal fat pads, hence reduced fat content of birds.

The study by Ni and Li (1998) have revealed that as a result of the action of EM, the utilisation coefficient and the transfer rate of the nitrogenous compounds in the foodstuff were increased. The dose of 1mlEM/l provided in drinking water improved the growth of poultry, increased egg production and the length of laying period. The egg production of some chicken were increased by 13%. For broilers, the rate of weight gain was faster, with better quality meat and efficiency of feed utilisation. The ratio of feed to meat production was reduced by 10.24 % and the economic benefit was raised by 18.41%. Similar observations were reported by Willis and Reid (2008) who found that live body weight gain and carcass yields were significantly higher in broilers supplemented with probiotics. In another study these authors found that there were improved egg size and lowered feed cost in laying hen which were supplemented by probiotics.

Tariq *et al* (2005) reported significant increase in digestibility of crude protein and crude fibre in digestibility and growth performance study of growing Rabbits supplemented with probiotic Lacto-sacc (Yeast culture & Lactic acid producing bacteria) which were added in pelleted diet at the rate of 1g Lacto-sacc/kg pelleted diet. Consequently there were increased growth performance of growing rabbits.

Likewise Sangakkara (2011) reported more successful use of EM in poultry and swine units, where EM was added to feed and sprayed for sanitation in these units. EM in pig's diet indicated the potential of using it for treating pig manure prior to feeding fish. Application of EM to manure reduced faecal bacterial counts and feeding this manure to fish increase harvestable produce. The author also noted that in Austria EM is used for Swine and fish units to improve productivity. The study of growing goats on the use of EM in goat diet showed that the body weight gain was higher in feed mixed with 10% EM Bokashi as compared to 5% and control (Bhola, 2011). However, Flint and Garner (2009) reported that there were no significant difference between body weight gain, feed conversion or chick quality compared with control groups. Various reasons were advanced for these observations including that the product had insufficient numbers of viable organisms, the product was improperly manufactured or it contained inappropriate organisms or impairment of bird performance by infectious agents that were not affected by the probiotic product applied.

2.7 Use of Probiotics for Diseases Control and Mortality in Poultry

Healthy birds are generally characterized by having a well functioning gastro intestinal tract (GIT). This is very important for the efficient feed conversion, for maintenance and for growth or production (Botlhoko, 2009). Intestinal bacteria have a profound effect on the immune development of the gastrointestinal system and are a major source of antigenic

material that stimulate the development of gut associated lymphoid tissue and Peyer's patches, the production of antimicrobial peptides and the production of protective IgA molecules (Flint and Garner, 2009).

To feed the animal with probiotics is to utilize the microbes to build and recover the microecological balance in the animal body. It avoids the use of antibiotics that destroy the microecological balance of the animal body so as to reduce the phylactic power. Therefore, the use of EM as feed additive result in the animal being healthy with high quality products with multiple benefits (Ni and Li, 1998).

Li *et al* (1998) reported that mortality of birds receiving 30mgEM/kg decreased more than 35 percent compared with control (non-EM treated birds). Also 30mgEM/kg fed to egg laying hen from 0 – 57 weeks, significantly decreased the incidences of contagious intestinal diseases such as bacillary white diarrhea. Botlhoko (2009) noted that the incidences of mortality for broiler chickens was as low as 2.2 percent and examination of livers and intestines showed only mild necrotic enteritis lesions when EM was added to feed and water at the rate of 50g/kg and 50ml/L respectively from one to forty days. The colonization of lactic acid bacteria in the chickens intestinal tract have apparently been shown to control the population of pathogenic microorganisms such as Salmonella species, *Enterococci* and *Escherichia coli* (SCD Probiotic, 2009).

2.8 The use of Probiotics for Odour Control in Poultry Production

2.8.1 The Odour Problem in Poultry Production

Odour is a major problem confronting confined livestock production systems. In poultry facilities some of the odour-causing compounds like ammonia are health hazards for people who work in the facility, for the animals and are also a nuisance for the

surrounding community. Due to the rising costs in both labour and materials, poultry farmers are re-using old litter for as long as four or five flocks. The result of this practice is a significant increase in the levels of ammonia inside and outside the chicken houses. The formation of ammonia has been attributed to the microbial decomposition of uric acid in the manure (SCD Probiotic, 2009).

The study on the effects of ammonia on poultry show that it adversely affects growth rates, feed efficiency, egg production, respiratory tract, susceptibility to Newcastle disease, incidence of airsacculitis, levels of *Mycoplasma gallisepticum*, and incidence of Keratoconjunctivitis. Therefore, it is recommend that ammonia levels in poultry houses should not exceed 25 ppm. In practice however, the birds are often exposed to levels of over 50 ppm to as high as 200 ppm. Humans detect ammonia levels above 25 ppm and exposure of 100 ppm for eight hours has acute adverse health effects (SCD Probiotic, 2009).

There are four different ways in which EM inoculants can be introduced into the production system in order to achieve a deodorizing effect. Those methods involves the use of EM as a probiotic additive to drinking water, as a probiotic feed additive, as an additive to sanitation spray water for washing the facility and as a treatment added to the waste handling process (SCD Probiotic, 2009).

The rate of ammonia volatilization and resulting ammonia concentration in a chicken house depends on factors such as litter moisture content, pH, temperature and wind speed (SCD Probiotic, 2009). The odour that is detected from a poultry operation is a complex mixture of gases. Most often the odour is a result of the uncontrolled anaerobic decomposition of manure. However, feed spoilage can also contribute to the odour. The

odour that our noses detect can be a combination of 60 to 150 different compounds. Some of the most important types of odour causing compounds are: volatile fatty acids, mercaptans, esters, carbonyls, aldehydes, alcohols, ammonia, and amines (Chastain, 2005). The odour strength of these compounds do not combine in an additive manner. That is, sometimes mixing several of these compounds can result in reduced odour by dilution of the strongest smelling compounds. In other instances, the mixture is worse than any of the individual compounds. Ammonia can create strong odours near the manure storage or building also is the largest contributor to foul odours being emitted from poultry facilities. Ammonia is highly volatile and moves upward in the atmosphere quickly where it is diluted (Chastain, 2005).

The improvement in poultry performance, among other things is due to the use of EM that leads to having healthier birds living in a healthier environment (SCD Probiotic, 2009). A study on effect of EM on malodour revealed that pens with broiler birds receiving 1mlEM/l had lower concentration of toxic Ammonia (26.5 ppm) compared with non-EM control groups 87.6 ppm (Li, *et al*., 1998). Further noted that, EM can effectively transform NH_3 into less toxic substances therefore maintaining a safer environment to birds. The authors further reported that when EM was applied in egg laying hens feed it resulted in a substantial reduction in the concentration of NH_3 and H_2S (hydrogen sulfide) which are toxic gases that are harmful and even lethal to animals. In these studies EM reduced the concentration of these gases inside poultry houses by 30 to 50 percent compared with non-EM control.

2.8.2 Mechanism for Probiotic in Odour Control

EM has multiple functions with excellent character which is closely related to the actions of the dominant species in EM such as photosynthetic bacteria, *saccharomyces*, lactic acid bacteria and *Actinomyces.* According to Ni and Li (1998) the mechanism for the control of foul odour might be due to the fact that:

(a) EM microbes have rather strong nitrogen fixing ability,

(b) EM contains beneficial microbes which enter into the animal intestines with feed and drinking water and then exclude the putrifying bacteria group in the intestines so as to check the activity of the intestinal *colibacillus* and transfer the protein into ammonia and ammonium,

(c) The EM in the intestines may be able to reduce the ammonia in the dung and blood.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Birds:

Two hundred and ten, day old Hybro unsexed (mixed males and females) broiler chicks, with mean live body weight of 34.7 ± 0.4 g, from Kibaha Education Centre in Pwani region were used in the experiment. These chicks were randomly allocated in 14 pens of 15 chicks each. The chicks were vaccinated against Newcastle and Gumboro diseases in the first week of their life. All hygienic procedures were observed and no antibiotic was intended to be used for all experimental birds for the whole experimental period of six weeks, in order to observe the efficacy of probiotics in disease control right from brooding and the rest of experimental period. However, in the first week antibiotic and coccidiostat were used due to the outbreak of coccidiosis and salmonellosis diseases. This is probably due to contamination from the hatchery unit. After the first week no antibiotic or coccidiostat were used.

3.1.2 Poultry House

Chickens were intensively reared in a closed well ventilated house, each pen measured 10ft x 5ft with a deep litter floor (Three Inch thick) of rice husks. During brooding period (from 1 to 14 days of age) supplementary heat was provided using 200w electric lamps (bulbs), which were placed in each pen, there after they were left under ambient room temperature.

3.1.3 Feeds

Commercial Broiler starter mash was fed as basal diet and was supplied from 1st to 21st day and Growers mash from 22nd to 42nd day. Both commercial feeds were purchased from

TANFEED INTERNATIONAL, animal feed suppliers in Morogoro municipality. The starter mash and growers mash had 21.03% CP and 19% CP respectively, on dry matter basis (Kjeldahl laboratory analysis, DASP). Feeds and water were given *ad libitum* for the whole experimental period of six weeks. Feeds were provided by using round metal feeders with the capacity of holding 2 kg during brooding and others carrying 4 kg were used after brooding period. Water was provided in round plastic drinkers that carries 2 L during brooding and 4 L after brooding period. Each pen was provided with two feeders and two drinkers for the whole experimental period.

3.1.4 Effective Microorganisms

EM-1 original stock solution, developed in Austria, was used as the main treatment. It contains Photosynthetic bacteria: *Rhodopseudomonas palustris*, Lactic acid bacteria: *Lactobacillus pluntarum* and *Lactobacillus casei*, Yeast: *Saccharomyces cerevisae*, Molasses and water, with pH 3.5. EM-1 is inactive solution, so to make it active more water and molasses were added as indicated in section 3.2. No percentages of composition were given by the EM-1 manufacturer.

3.1.5 Litter Materials

Rice husks were used as litter materials, which were spread in all pens with a layer of three inches thick. Rice husks were obtained from rice/cereal milling machines found in Morogoro municipality.

3.2 Preparation of EM-2 Solution

One litre EM-1, One litre Molasses and 18 litres of clean water were used to prepare EM-2 solution. The ingredients were mixed as follows:

a) First step involved taking one litre of molasses which was mixed with five litres of warm clean water which was free from chlorine. The mixture was stirred thoroughly then it was poured in a clean plastic container with 20 litres capacity.

b) Second step involved mixing one litre of EM-1 with a mixture of molasses and water obtained in (a). The remaining part of the container (i.e. 13 litres) was filled with clean water to make 20 litres EM-2 and the container was tightly covered with a lid. Finally the 20 litres of EM-2 solution contained one litre of EM-1, one litre molasses and 18 litres water. The solution was kept in a place with an ambient room temperature ranging between 22°C and 30°C for 10 days before being used.

c) Third step, was to ascertain the properties of EM-2 solution if it is ready to be used. Therefore, after ten days the solution was observed visually and it appeared yellowish-brown in colour with characteristic sweet/pleasant smell and had sour test. The pH of the resulting solution was 3.54 after 10 days. The appropriate pH range of EM-2 is supposed to be between 3 and 3.8. Those properties indicated that the solution was ready to be used. The prepared solution was valid for 30 days. Note: All steps above are as per manufacturer's instructions.

3.3 Treatment Application

Effective Microorganisms (EM-2) i.e. main treatment, was allocated randomly to experimental birds by mixing it in drinking water and sprayed in litter materials into two levels; 10ml EM2/litre of drinking water and 20ml EM2/litre of drinking water. Litter material were sprayed with the same rate of concentration that have been indicated for drinking water. There were seven treatments and each treatment was replicated twice.

3.3.1 Experimental Treatments

Seven treatments were formulated as follows:

(T1) 0ml EM2/l: birds were fed basal diet only *ad libitum* without EM2 (control). Basal diets were commercial broiler starter mash and broiler growers mash.

3.3.2 Treatment Level One, 10ml EM-2/litre of water

(T2) 10ml EM2/l Sprayed in litter materials only, once a week with basal diet *ad libitum.*

(T3) 10ml EM2/l mixed with drinking water only, daily with basal diet *ad libitum.*

(T4) 10ml EM2/l mixed with drinking water daily and sprayed in litter materials once a week with basal diet *ad libitum.*

3.3.3 Treatment Level Two, 20ml EM-2/litre of water

(T5) 20ml EM2/l Sprayed in litter only, once a week with basal diet *ad libitum.*

(T6) 20ml EM2/l mixed with drinking water only, daily with basal diet *ad libitum.*

(T7) 20ml EM2/l mixed with drinking water daily and sprayed in litter once a week with basal diet *ad libitum.*

3.4 Parameters Measured and Data Collection

3.4.1 Growth Performance

Body weight of all birds were measured in the first day, as initial weight just on arrival and subsequent body weight to the nearest two decimal points in grams were taken on weekly basis for six weeks using digital weighing balance. Live body weight measuring and recording were performed early in the morning, between 0600h and 0800h. Overall body weight gain was determined as the difference between final body weight and initial weight. Cumulative weight gain was derived by adding subsequent weekly gains, minus initial weight.

Daily weight gain of the birds were derived as follow:

$$W = (Wt2 – Wt1)/(T2 – T1) \quad \text{……..(1).}$$

Where:

W = Average daily gain in grams per day/bird

Wt1 = Live body weight at time 1

Wt2 = Live body weight at time 2

T1 & T2 are time intervals.

3.4.2 Carcass Evaluation

At the end of experiment (42 days) 10 birds from each treatment (i.e. 5 birds per pen) were randomly selected and slaughtered to evaluate carcass weight, carcass yield, gizzard and liver weights. Each live bird intended for slaughter was identified, live body weights were individually weighed and recorded. After slaughter all components of each bird were carefully labeled in reference to the identity of respective live bird. Dressed (plucked) carcass weight and carcass yield were also individually measured. Internal organs particularly crop and gizzard were weighed after emptying (removing the contents) also hearts and livers were weighed individually using digital weighing balance. Carcass yields were calculated as percentages of dressed (plucked) carcass weight and analysed for significant difference between treatments .

3.4.3 Disease and Mortality Rates

Feeding behavior, respiratory behavior and faecal material of all birds were visually observed daily to ascertain general health status of experimental birds. Bodies of 27 birds found dead were disposed by incineration among which 13 bodies were submitted to the

Department of Veterinary Pathology, Sokoine University of Agriculture for pathological examination

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3.4.4 Testing Ammonia Concentration in Litter

One sample of litter materials from each replicate (pen) i.e. a total of 14 samples were collected and tested in the laboratory at two weeks interval for ammonia concentration. Samples were collected early in the morning between 0600h and 0700h.

Three samples from each pen were randomly collected from different location and then mixed thoroughly so as to have a homogeneous representative mixture of litter sample. Thereafter 50g were weighed and placed in 500cc sampling bottle. Five hundred milliliters of clean distilled water were added to each sample to dissolve the sample. Dissolved and filtered litter fluids were determined for ammonia-N concentration using Kjeldahl technique without digestion. It was assumed that ammonia (NH_3) in litter material was free. Therefore 5ml litter fluid + Sodium hydroxide (NaOH) was distilled into boric acid using Kjeltec apparatus and blank distillation were carried out. The blank was 5ml of distilled water + NaOH. The litter fluid and blank distillate were titrated against 0.1M HCl to estimate N content, hence NH_3 concentration was calculated according to Doto (2002) using the following equation:

Results are given in concentration of NH3mg/l or NH3ppm (Table 7)

$$NH_3\ (mg/l) = \frac{N \times (V_{litter\ fluid} - V_{blank}) \times \text{molecular weight of } NH_3 \times 100}{\text{Volume of Litter Fluid}} \quad \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Where: NH_3(mg/l) = Concentration of ammonia.

N = Normality of HCl, acid used during titration.

V = Volume of HCl, acid used in litter fluid or blank titration.

3.5 Statistical analysis

The experiment was tested by using factorial design in which nesting effects, interaction effects and treatment effects were tested for all parameters. Growth performance, Carcass evaluation and Ammonia concentration data were analysed using the General linear model procedure of SAS version 8 (V8) in accordance to the following statistical models:

Model 1 : General model (Includes pens with no EM)

$Y_{ijk} = \mu + T_i + (P_i)_j + \epsilon_{ijk}$ …….. (3)

Where: Y_{ijk}= Observation on i^{th} treatment and j^{th} replication

μ = General mean

T_i = Effect of i^{th} concentration (1= 10ml/l, 2= 20ml/l)

$(P_i)_j$ = Effect of j^{th} replication within i^{th} treatment

ϵ_{ijk} = Random effect peculiar to each bird.

Model 2 : Evaluation of effect of treatment combination

$Y_{ijkl} = \mu + C_i + M_j + (CM)_{ij} + (P_{ij})_k + \epsilon_{ijkl}$ …….(4)

Where : Y_{ijkl} = Observation i^{th} concentration, j^{th} medium and k^{th} replication

μ = General mean

C_i = Effect of i^{th} concentration (1 = 10ml/l, 2 = 20ml/l).

M_j = Effect of j^{th} medium (1 = litter, 2 = water).

$(CM)_{ij}$ = Interaction between j^{th} medium and i^{th} concentration

$(P_{ij})_k$ = Effect of k^{th} replication within i^{th} concentration and j^{th} medium

ϵ_{ijkl} = Random effect peculiar to each bird

Model 3: After testing the above two proposed models, there were very small or no interaction effects and no nesting effects in most cases. Therefore, the following model was adopted to test treatments effects for growth performance, carcass weight, carcass yield and ammonia concentration.

$Y_{ij} = \mu + T_i + \epsilon_{ij}$……(5)

Where: Y_{ij} = Observation on i^{th} treatment

μ = General mean

T_i = Effect of i^{th} treatment (1 = 10ml/l, 2 = 20ml/l).

ϵ_{ij} = Random error

CHAPTER FOUR

4.0 RESULTS

4.1 Growth Performance

Least square means for treatment effects on broiler growth performance are shown in Table 1. The results shows that there were significant differences between treatments. Coefficient of determination in all models were generally high. Birds receiving EM in water + litter (T7) at the rate of 20mlEM/l tended to be heavier (1658.6g, 1624g and 38.7g) in final body weight, cumulative body weight and average daily weight gain respectively than birds in the control (T) group and other treatments.

Furthermore, the results in Table 2 shows that there were significant differences between treatment combinations while there was no significant interaction effects for litter + water and EM levels. However, birds receiving 20mlEM/l in water (T6) revealed higher least square means (1576.9g, 1542.3g and 36.7g) for final body weight, cumulative weight gain and average daily weight gain respectively, than birds receiving similar level of EM (sprayed) in litter only.

Table 1: Least square means for treatment effect of EM on growth performance for broiler chickens.

Treatment			Parameter			
EM Levels	**No.**	**Medium**	**Initial body weight (g)**	**Final body Weight (g)**	**Cumulative weight gain (g)**	**Average daily weight gain (g)**
0mlEM/l	**T1**	**Control**	34.6^{a} ±0.4	1207^{f} ±9.1	1172.4^{f} ±9.1	27.9^{f} ±0.2
10mlEM/l	**T2**	**Litter**	34.8^{a} ±0.4	1260.8^{e} ±9.2	1226.1^{e} ±9.2	29.2^{e} ±0.2
	T3	**Water**	34.6^{a} ±0.4	1339.8^{d} ±9.1	1305.1^{d} ±9.1	31.1^{d} ±0.2
	T4	**Litter + water**	34.8^{a} ±0.4	1372.6^{d} ±9.1	1337.9^{d} ±9.1	31.9^{d} ±0.2
20mlEM/l	**T5**	**Litter**	34.7^{a} ±0.4	1406.8^{c} ±9.1	1372.1^{c} ±9.1	32.7^{c} ±0.2
	T6	**Water**	34.4^{a} ±0.4	1495.5^{b} ±9.5	1460.8^{b} ±9.4	34.8^{b} ±0.2
	T7	**Litter + water**	34.9^{a} ±0.4	1658.6^{a} ±9.2	1623.9^{a} ±9.2	38.7^{a} ±0.2
R- square			99.7	99.7	99.7	99.7

^{abcdef} means with different superscripts within the same column are significantly different ($P<0.05$).

Table 2: Least square means for effect of EM for treatment combinations on broiler chickens growth performance.

		EM Levels				
Parameter	**Medium**	**10mlEM/l**		**20mlEM/l**		**R-square**
		No EM	**With EM**	**No EM**	**With EM**	
Final body weight	**Litter**	1283.8^{c} ±11.2	1316.9^{c} ±6.5	1368.9^{b} ±11.7	1532.9^{a} ±6.5	99.6
	Water	1244.5^{c} ±11.2	1356.2^{b} ±6.5	1324.8^{b} ±11.4	1576.9^{a} ±6.5	
Cumulative weight gain	**Litter**	1249.1^{c} ±11.2	1282.2^{c} ±6.5	1334.2^{b} ±11.7	1498.2^{a} ±6.5	99.5
	Water	1209.8^{c} ±11.2	1321.5^{b} ±6.4	1290.1^{b} ±11.4	1542.3^{a} ±11.4	
Average daily wt g	**Litter**	28.8^{c} ±0.3	30.5^{c} ±0.2	31.8^{b} ±0.3	35.7^{a} ±0.2	99.6
	Water	28.8^{c} ±0.3	31.5^{b} ±0.2	30.7^{b} ±0.3	36.7^{a} ±0.2	

^{abc} means with different superscripts within the same row are significantly different ($P<0.05$).

Note: No EM indicates that EM was provided in the alternative medium, that is, if No EM in litter then it was in water and vice versa.

4.2 Carcass Evaluation

Least square means for treatment effect on carcass yield and weight of organs are shown in Table 3. The results showed that there were significant differences between treatments. The treatment effects show that higher values were obtained from birds receiving EM in water + litter (T7) at the rate of 20mlEM/l for the carcass yield (1121.1g), gizzard weight (46.6g) and liver weight (39g) than other treatment groups, while control group had the lowest values for the three parameters.

Least square means for treatment combinations on carcass yield, gizzard and liver weight are shown in Table 4. There were significant differences in treatment combinations. However there were no significant interaction effects for litter + water and EM levels, also no significant nesting effect was found in all models for replication within treatment and media. The results for treatment combinations shows that, birds raised in litter treated with 20mlEM2/l had higher carcass yield 1125.5g compared with other treatments. Birds receiving 20mlEM/l in water had relatively high values of 43.3g and 37g for gizzard weight and liver weight respectively. Nonetheless, the difference between Gizzard weight and liver weight in birds receiving 10mlEM2/l and those supplied with 20mlEM2/l did not differ significantly.

Table 3:Least square means for treatment effects of EM on carcass yield, Gizzard weight and Liver weight.

Treatment			Parameter		
EM Levels	**No.**	**Medium**	**Carcass Yield (g)**	**Gizzard Weight (g)**	**Liver Weight (g)**
0mlEM/l	**T1**	**Control**	1036.8^{e} ±11.55	32.8^{c} ±2.2	29.1^{c} ±1.9
10mlEM/l	**T2**	**Litter**	1060.9^{d} ±11.1	42.5^{ab} ±2.1	34.9^{ab} ±1.8
	T3	**Water**	1067.4^{bc} ±11.1	40.1^{b} ±2.1	32.6^{bc} ±1.8
	T4	**Litter +water**	1093.9^{c} ±11.1	38.9^{bc} ±2.1	35.1^{ab} ±1.8
20mlEM/l	**T5**	**Litter**	1090.1^{bc} ±11.1	38.9^{bc} ±2.1	30.7^{bc} ±1.8
	T6	**Water**	1076.9^{b} ±11.2	40.1^{b} ±2.1	34.9^{ab} ±1.9
	T7	**Litter + water**	1121.1^{a} ±11.3	46.6^{a} ±2.2	39^{a} ±1.9

^{abcde} means with different superscripts within the same column are significantly different ($P<0.05$).

Table 4:Least square means for the effect of EM for treatment combinations on carcass yield, gizzard weight and Liver weight.

Parameter	Medium	EM Levels (Concentration)			
		10mlEM/l		20mlEM/l	
		No EM	With EM	No EM	With EM
Carcass yield (g)	**Litter**	1071.3^{a} ±11.4	1098.8^{b} ±6.6	1082^{ab} ±11.4	1125.5^{c} ±6.6
	Water	1068.6^{c} ±11.4	1101.6^{ab} ±6.6	1088.9^{bc} ±11.4	1118.6^{a} ±6.6
Gizzard weight (g)	**Litter**	41.9^{ab} ±2.8	40.7^{abc} ±1.6	36.2^{c} ±2.8	42.8^{a} ±1.6
	Water	43.1^{ab} ±2.8	39.5^{abc} ±1.6	35.7^{c} ±2.8	43.3^{a} ±1.6
Liver weight (g)	**Litter**	32.6^{a} ±2.4	35^{a} ±1.4	30.8^{a} ±2.4	34.9^{a} ±1.4
	Water	33.7^{abc} ±2.4	33.9^{ab} ±1.4	28.7^{c} ±2.4	37^{a} ±1.4

^{abc} means with different superscripts within the same row are significantly different ($P<0.05$).

Note: No EM, indicates that EM was provided in the alternative medium, that is, if No EM in litter then it was in water and vice versa.

Table 5 Presents the results for carcass yield percentages. Carcass yield as the percentage of carcass (plucked) dressed weight was higher (79.1%) in group of birds receiving EM in water + litter (T7) at the rate of 20mlEM/l compared to control (T1) group (73.9%) and other treatments. Generally birds in control group had the lowest value of carcass yield percentage than other treatment groups.

4.3 Mortality Rate

The results of mortality rates of birds for the whole experimental period are presented in Table 6. The results showed that a total of 27 birds (12.8%) died and high mortality rates occurred in the first week when 19 chicks (9%) died. In the second week seven birds (3.3%) died and only one bird (0.5%) died in the fourth week. There were no mortalities in the third, fifth and sixth weeks. Birds receiving 20mlEM2/l in litter (T5) and control (T1) groups had high mortalities, (20% in each group on treatment basis), compared to other groups. The lowest mortality percentages occurred in birds received EM in water (T3) and water + litter (T4) at the rate of 10mlEM/l which had 1% mortalities for each treatment.

The investigation results suggested that the cause of death were sudden death syndrome 5 birds, coccidiosis disease 8 birds, salmonellosis disease 6 birds, vitamin B1 (Thiamine) deficiency 1 bird and 7 birds died due to crowding/huddling as a result of low temperatures caused by unexpected electric power cuts during the nights.

Table 5: Carcass yield as percentages of dressed (plucked) carcass weight.

Treatment			Parameter		
EM Levels	**No.**	**Medium**	**Carcass dressed weight (g)**	**Carcass Yield (g)**	**Carcass Yield %**
0mlEM/l	**T1**	**Control**	1402.7^{c} ±5.7	1036.8^{e} ±11.6	73.9
10mlEM/l	**T2**	**Litter**	1402.3^{c} ±5.5	1060.9^{d} ±11.1	75.7
	T3	**Water**	1400.2^{c} ±5.5	1067.4^{bc} ±11.1	76.2
	T4	**Litter + water**	1403.3^{c} ±5.5	1093.9^{c} ±11.1	77.9
20mlEM/l	**T5**	**Litter**	1411.9^{b} ±5.5	1090.1^{bc} ±11.1	77.2
	T6	**Water**	1390.2^{c} ±5.5	1076.9^{b} ±11.2	77.5
	T7	**Litter + water**	1416.8^{a} ±5.5	1121.1^{a} ±11.3	79.1

^{abcde} means with different superscripts within the column are significantly different ($P<0.05$).

Table 6 Number and mortality rate of birds during the experimental period.

TREATMENT			LIVE	DIED IN WEEKS						TOTAL		
EM Levels	**No**	**EM in Medium**	**Chicks**	**1**	**2**	**3**	**4**	**5**	**6**	**Chicks Died**	**Survived**	**% mortalities/treatment**
0mlEM/l	**T1**	**Control**	30	3	2	0	1	0	0	6	24	20
10mlEM/l	**T2**	**Litter**	30	3	1	0	0	0	0	4	26	13.3
	T3	**Water**	30	2	0	0	0	0	0	2	28	6.6
	T4	**Litter + Water**	30	2	0	0	0	0	0	2	28	6.6
20mlEM/l	**T5**	**Litter**	30	3	3	0	0	0	0	6	24	20
	T6	**Water**	30	2	1	0	0	0	0	3	27	10
	T7	**Litter + Water**	30	4	0	0	0	0	0	4	26	13.3
Total			210	19	7	0	1	0	0	27	183	
Weekly, % mortality				9	3.3	0	0.5	0	0			12.8

4.4 Ammonia Concentration in Litter

Least square means for the treatment effects on ammonia concentration are shown in Tables 7. There were significant differences between treatment effects. Pens in which birds were receiving 20mlEM2/l in water + litter (T7) had low Ammonia levels of 28mg/l, 26mg/l and 22mg/l for the second, fourth and sixth weeks respectively, compared with the control group (T1) which had 46.1mg/l, 50.1mg/l and 70.1mg/l in the same period, that is, for second, fourth and sixth weeks respectively. However, in the second week, pens in T6 and T7 had lower ammonia levels compared with control and other treatments.

There were no significant difference between T3, T4, T5, T6 and T7 in the fourth week, yet T7 still had low ammonia concentration. Ammonia concentration in the control treatment increased by 8% in the fourth week and 34% by end of sixth week.

Least square means for treatment combinations are shown in Table 8. There were significant difference between treatment combinations. The results shows that, for whole experimental period, low levels of ammonia were obtained in group of birds receiving 20mlEM2/l either in litter or in water. However, lowest value was obtained in the sixth week in birds receiving 20mlEM/l in water.

The trend of Ammonia concentration in litter for the experimental period is presented in Figure 1. The trend show that there were progressive increase of Ammonia concentration in control group in the course of time during experiment. The general trend was for ammonia to decrease in the remaining treatments. By sixth week the lowest concentration was in T7 but values did not differ significantly with those of T5 and T6.

Table 7: Least square means for treatment effects for Ammonia (NH3) concentration in litter materials.

Treatment			NH_3 mg/l (weeks)		
EM Levels	**No.**	**EM in Medium**	**2nd**	**4th**	**6th**
0mlEM/l	**T1**	**Control**	46.1[a]	50.1[a]	70.1[a]
10mlEM/l	**T2**	**Litter**	40.1[b]	38.1[b]	34[b]
	T3	**Water**	38.1[bc]	32[bc]	30[bc]
	T4	**Litter +water**	34[bc]	30[bc]	28[bc]
20mlEM/l	**T5**	**Litter**	36.1[cd]	30[bc]	26[cd]
	T6	**Water**	30[de]	28[c]	24[cd]
	T7	**Litter +water**	28[e]	26[c]	22[d]
Standard error of the mean			±1.6	±2.4	±1.8

[abcde] means with different superscripts within the same column are significantly different ($P<0.05$).

Table 8: Least square means for treatment combinations for Ammonia (NH_3) concentration in litter materials.

Sampling & testing time	Medium	EM Level			
		10mlEM/l		20mlEM/l	
		No EM	With EM	No EM	With EM
2nd week	Litter	41.1[a] ±1.7	37.1[ab] ±1.0	34.1[bc] ±1.7	32.1[c] ±1.0
	Water	42.1[a] ±1.7	36.1[b] ±1.0	37.1[ab] ±1.7	29[c] ±1.0
4th week	Litter	36.1[a] ±2.8	34.1[a] ±1.6	30[ab] ±2.8	28[b] ±1.6
	Water	39.1[a] ±2.8	31[ab] ±1.6	31[ab] ±2.8	27[b] ±1.6
6th week	Litter	33[a] ±2.0	31[ab] ±1.2	26[bc] ±2.0	24[c] ±1.2
	Water	35.1[a] ±2.0	29[b] ±1.2	27[bc] ±2.0	23[c] ±1.6

[abc] means with different superscripts within the same row are significantly different ($P<0.05$).

Note: No EM, indicates that EM was provided in the alternative medium, that is, if No EM in litter then it was in water and vice versa.

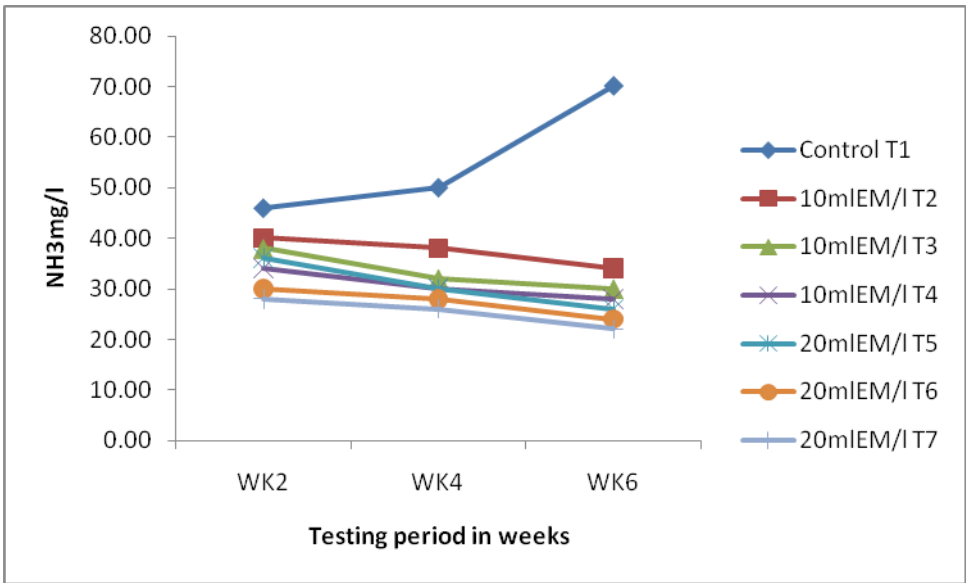

Figure 1: The trend of Ammonia concentration in litter materials during experimental period.

CHAPTER FIVE

5.0 DISCUSSION

5.1 Effect of EM on Growth Performance

The results show clearly that supplementing EM in drinking water and spraying in litter materials concurrently, stimulated the growth of broiler chickens during the entire experimental period compared to non-EM control group. EM supplemented birds were heavier than control group. Related results were reported by Bozkurt *et al,* (2009), Willis and Reid, (2008), Ashayerizadeh *et al*, (2009) and Ashraf *et al,* (2005). The improved body weight gain were reflected in carcass yield in which, relatively high values were observed in EM treated birds than non-EM control group. Shareef and Al-Dabbagh (2009) reported that the growth promoting effect of probiotics (particularly *Saccharomyces cerevisiae*) is attributed to the growth improvement, due to the fact that it is a naturally rich source of protein, minerals and Vitamin B-complex. The greater physiological activity in animals and better feed conversion efficiencies may be attributed to improved growth performance for broilers and use of probiotics has also been shown to improve protein efficiency ratios and/or nitrogen utilisation in broilers as reported by Weijiong and Yongzhen (2001) and Safalao (2006).

Therefore, this study suggests that EM fed broilers were more efficient at converting feed to body mass during the entire experimental period. This result supports the study by Bozkurt *et al,* (2009) who noted that improvements in feed efficiency were attributed to an encouraged growth of the beneficial micro flora in the gastrointestinal tract induced by dietary supplementation of probiotics. The author further reported that there was significantly increased intestinal amylase enzyme activity when adding *Lactobacilus acidophilus* and a mixture of *Lactobacilli* to the diets. Furthermore, reported that the

improvement in feed efficiency of birds receiving probiotic supplemented diets could be due to decreased urease activity in the gastrointestinal tract of the broiler chicks.

According to this study the preferred EM dose rate is 20mlEm/l supplemented in drinking water and spraying in litter materials. At this dose rate (T7), similar trend of body weight, cumulative and average body weight gain were obtained and there were at the highest levels compared with other treatments. This suggests that there is dose dependency of EM, in order to have optimum required growth rate, as reported by Flint and Garner (2009) and Safalao and Smith (2002) that addition of EM at the rate of 15g/kg of feed elicited no beneficial effects on body weight gain while an inclusion rate of 30g/kg of EM with or without antibiotic resulted in improved body weight gain.

5.2 Carcass and Internal Organs Characteristics

The high carcass yield percentages, gizzard weight and liver weight observed in this study for birds treated with 20mlEM2/l in litter + water (T7) compared to control(T1) and other treatment is related to high body weight observed to the same treatment (Table 1). Similar results were reported by Safalao and Smith (2002). This suggests the beneficial effects of EM products in improving carcass yield and giblet yield. Having a high carcass yield percentage of 79.1% in (T7) against 73.9% in control group is the most significant characteristic required by poultry producer and meat consumers. In economic terms, higher carcass yield as observed in T7 against control translates into more financial returns.

It is interesting to note, in this study that there were no significant differences between treatment effects (Table 5) in control (T1) group and other treatments (T2, T3, T4 and T6) for dressed (plucked) carcass weight, while control group were significantly different compared with other treatments for carcass yield weight. One of the explanation may be

that, the active constituents of probiotics, when fed to broilers, played an important role in improving quantitative carcass traits, that is, EM improved more carcass components other than non-edible components.

Although feed conversion efficiency was not measured in the present study, there is likelihood that inclusion of EM in litter and water could have a positive effect on feed utilisation, as reported by Flint and Garner (2009) who noted that supplementing EM to poultry demonstrated increased body weight gain and greater feed conversion efficiency.

5.3 Mortality Rate

Results shows that 12.8% of the total experimental birds died. Significant death rate of chicks mainly occurred in the first week, 9% and second week 3.3% died, basically this is the brooding period. The main causes of death were sudden death syndrome and salmonellosis as reported by the Pathologist in the Department of Veterinary Pathology, Sokoine University of Agriculture. The source of contamination may be from the hatchery unity. However, during brooding, the period was associated with unexpected intermittent power cuts, especially during the nights. This suggests that some of the chicks may have died due to huddling and crowding due to low brooding temperatures at nights. During the early stage of life chicks are very prone to diseases, low and high temperatures.

It was intended that, no coccidiostats and antibiotic could be used for the whole experimental period, but it was felt necessary to use coccidiostats and antibiotic to contain the situation during the brooding period (first 10 days). No coccidiostats or antibiotic were used after first ten days. Chicks were vaccinated against Newcastle disease in the first week and gumboro disease in the second week. No further death occurred after the second week, except that, only one bird died in the fourth week in no-EM control group with

signs of vitamin B1 (Thiamine) deficiency. This suggests that probiotics were not potentially efficient in controlling diseases in the early stage of chicks life particularly during brooding period. Although no more birds died after fourth week in no-EM control group, it suffice to assume that just after brooding period probiotics started to be potentially active in disease control. This explains the ability of probiotics to beneficially stimulate poultry immune system as reported by Dharne (2008), Anjum *et al* (2011), Ashraf *et al* (2005), Dunkley (2008), Gauthier (2006) and Patterson and Burkholder (2003). Further SCD Probiotic (2009) reported that Lactic acid bacteria produce significant amounts of bacterial growth inhibitory substances such as reuterin. Reuterin has a broad-spectrum antimicrobial activity that has proven to inhibit the growth of bacteria, fungi and protozoa. Furthermore Botlhoko (2009) reported that feeding broilers with probiotics helps to maintain beneficial intestinal microflora and may modulate the mucosal immune system and enhance the host resistance to enteric pathogens.

5.5 Ammonia Concentration in Litter Materials

The results of this study showed that there were progressive reduction of ammonia levels in litter materials for probiotics supplemented birds and the lowest levels were observed at the rate of 20mlEM/l in Litter + water (Table 7). On the other hand in the control group birds, there were progressive increase of ammonia concentration in litter materials as at the end of experiment the concentration of ammonia was 70.1mg/l. Similar observations were reported by Bhola (2011). The trend and pattern of these results for each treatment levels are clearly shown in Figure 1. Ammonia is an oxidative substance, it is putrefactive and supports the growth of pathogenic microbes. The decreasing patterns of ammonia concentration observed, indicates the ability of probiotics to suppress malodours in poultry houses. This is attributed by the efficiency of probiotics to transform NH_3 into less toxic substances. Teraganix (2011) reported that probiotics have an antioxidant effect hence

resists putrefaction and control pathogen growth. Weijiong and Yongzhen (2001) further reported that EM may transfer the protein in the feed into effective nutrients so as to raise the feed utilisation rate and check putrification which causes foul smell. In this regard EM may be considered to function as an "anti-oxidant".

The level of ammonia concentration observed in (T7) measured 22.03mg/l is within the safety level recommended by SCD Probiotics (2009) that in poultry houses ammonia levels should not exceed 25ppm (i.e. 25mg/l). Teraganix (2011) recommended that the average poultry house should have about 20-29ppm (i.e. 20-29mg/l) of ammonia.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

This study have demonstrated that supplementing probiotics in drinking water and spraying in litter materials has growth promoting effects. Birds receiving probiotics generally had increased final body weight, increased cumulative body weight gain, increased daily weight gain, and increased carcass yield percent compared with the control group. According to this study it can be concluded that probiotics had beneficial effects to broiler chickens performance, since they worked as safe natural growth promoters.

Moreover, the study have shown that there were improved health of probiotics supplemented broilers because no mortality occurred after brooding period. This shows that probiotics are less effective in suppressing diseases during brooding period when chicks have low immunity. The reduced chickens mortality after brooding period is potentially as a result of probiotics to boost immune system.

Furthermore, this study have demonstrated that probiotics significantly reduced ammonia levels in poultry house. Ammonia levels in probiotics treated birds were generally lower particularly at the rate of 20mlEM/l in drinking water and spraying in litter than the control group.

6.2 Recommendations

The use of probiotics for broiler production in Tanzania is a new technology. This study did not exhaust much of the aspects about the effects of EM for broiler chickens production due to lack of resources and time. Therefore, further research is needed to justify the economic benefits, feed conversion efficiency, growth performance, malodour

and disease control in different localities in Tanzania using different EM dosage levels for longer periods.

Probiotics are live natural microorganisms, therefore great care should be taken when using them as feed additives in order to attain optimum results in broiler chicken production.

The potential of EM in broiler production could be beneficially exploited and carefully, systematically introduced in the field to the farmers so that they may be able to produce natural organic broiler meat and realize optimum production hence increased profit.

According to the findings of this study the dose rate of 20mlEM/l supplemented in drinking water daily and spraying in litter materials weekly can practically be used to attain optimum performances in broiler chickens production in Tanzania.

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APPENDICES

Appendix 1: **Treatment effects for growth performance**

Appendix 1a. Analysis of variance (ANOVA) for final body weight

Dependent Variable: FNWT

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	6	272085.6722	45347.6120	275.78	<.0001
REP	1	265.7576	265.7576	1.62	0.2595
INWT	1	1.8866	1.8866	0.01	0.9189

R-Square	Coeff Var	Root MSE	FNWT Mean
0.997003	0.921470	12.82310	1391.591

Appendix 1b: Analysis of variance (ANOVA) for cumulative body weight gain

Dependent Variable: CUMWTG

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	6	272085.3847	45347.5641	275.89	<.0001
REP	1	265.8440	265.8440	1.62	0.2594
INWT	1	0.0116	0.0116	0.01	0.9936

R-Square	Coeff Var	Root MSE	CUMWTG Mean
0.997003	0.944843	12.82072	1356.915

Appendix 1c: Analysis of variance (ANOVA) for average daily weight gain

Dependent Variable: ADWTG

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	6	154.2210340	25.7035057	278.13	<.0001
REP	1	0.1470617	0.1470617	1.59	0.2628
INWT	1	0.0000006	0.0000006	0.01	0.9981

R-Square	Coeff Var	Root MSE	ADWTG Mean
0.997027	0.940974	0.304002	32.30714

Appendix 2 : **Treatment combinations for growth performance**

Appendix 2a: Analysis of variance (ANOVA) for final body weight

Dependent Variable: FNWT

Source	DF	Type III SS	Mean Square	F Value	Pr > F
CONC	1	43905.80359	43905.80359	264.14	<.0001
LITTER	1	17554.34582	17554.34582	105.61	0.0001
WATER	1	65928.50661	65928.50661	396.63	<.0001
CONC*LITTER	1	8351.63630	8351.63630	50.24	0.0009
CONC*WATER	1	9743.96280	9743.96280	58.62	0.0006
Inwt	1	1.21034	1.21034	0.01	0.9353

R-Square	Coeff Var	Root MSE	FNWT Mean
0.995726	0.906420	12.89265	1422.371

Appendix 2b: Analysis of variance (ANOVA) for cumulative body weight gain

Dependent Variable: CUMWTG

Source	DF	Type III SS	Mean Square	F Value	Pr > F
CONC	1	43904.31773	43904.31773	264.20	<.0001
LITTER	1	17554.37632	17554.37632	105.64	0.0001
WATER	1	65930.33087	65930.33087	396.75	<.0001
CONC*LITTER	1	8351.64698	8351.64698	50.26	0.0009
CONC*WATER	1	9744.66462	9744.66462	58.64	0.0006
Inwt	1	6.67705	6.67705	0.04	0.8490

R-Square	Coeff Var	Root MSE	CUMWTG Mean
0.995727	0.928960	12.89094	1387.675

Appendix 2c: Analysis of variance (ANOVA) for average daily weight gain

Dependent Variable: ADWTG

Source	DF	Type III SS	Mean Square	F Value	Pr > F
CONC	1	24.91178882	24.91178882	267.04	<.0001
LITTER	1	9.94516266	9.94516266	106.61	0.0001
WATER	1	37.37838464	37.37838464	400.68	<.0001
CONC*LITTER	1	4.74007767	4.74007767	50.81	0.0008
CONC*WATER	1	5.50799373	5.50799373	59.04	0.0006
Inwt	1	0.00331014	0.00331014	0.04	0.8580

R-Square	Coeff Var	Root MSE	ADWTG Mean
0.995769	0.924451	0.305431	33.03917

Appendix 3: **Treatment effects for carcass evaluation**

Appendix3 a: Analysis of variance (ANOVA) for Dressed (plucked) carcass weight

Dependent Variable: DWT

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	6	4331.152	721.859	2.43	0.0370
REP(TRT)	7	1487.912	212.559	0.72	0.6582
BWT	1	2755117.842	2755117.842	9288.34	<.0001

R-Square	Coeff Var	Root MSE	DWT Mean
0.994934	1.226762	17.22269	1403.914

Appendix3 b: Analysis of variance (ANOVA) for carcass yield

Dependent Variable: CWT

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	6	40603.306	6767.218	5.48	0.0002
REP(TRT)	7	6963.998	994.857	0.81	0.5864
BWT	1	1894792.522	1894792.522	1533.94	<.0001

R-Square	Coeff Var	Root MSE	CWT Mean
0.972323	3.259875	35.14610	1078.143

Appendix3 c: Analysis of variance (ANOVA) for Gizzard weight

Dependent Variable: GIWT

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	6	958.7812358	159.7968726	3.52	0.0051
REP(TRT)	7	251.6449596	35.9492799	0.79	0.5977
BWT	1	0.5380317	0.5380317	0.01	0.9137

R-Square	Coeff Var	Root MSE	GIWT Mean
0.338176	16.84749	6.740200	40.00714

Appendix3 d: Analysis of variance (ANOVA) for Liver weight

Dependent Variable: LIWT

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	6	606.7052553	101.1175425	2.99	0.0135
REP(TRT)	7	424.6397015	60.6628145	1.79	0.1076
BWT	1	6.7988262	6.7988262	0.20	0.6559

R-Square	Coeff Var	Root MSE	LIWT Mean
0.372278	17.23717	5.819761	33.76286

Appendix 4: **Treatment combinations for carcass evaluation**

Appendix 4a: Analysis of variance (ANOVA) for dressed (plucked) carcass weight

Dependent Variable: DWT

Source	DF	Type III SS	Mean Square	F Value	Pr > F
CONC	1	0.710	0.710	0.01	0.9627
LITTER	1	2210.576	2210.576	6.87	0.0117
WATER	1	84.667	84.667	0.26	0.6104
REP	1	10.598	10.598	0.03	0.8568
CONC*LITTER	1	1347.898	1347.898	4.19	0.0461
CONC*WATER	1	36.170	36.170	0.11	0.7389
REP(CONC*WATER)	3	799.628	266.543	0.83	0.4849
BWT	1	2560705.940	2560705.940	7954.59	<.0001

R-Square	Coeff Var	Root MSE	DWT Mean
0.994256	1.255695	17.94200	1428.850

Appendix 4b: Analysis of variance (ANOVA) for carcass yield

Dependent Variable: CWT

Source	DF	Type III SS	Mean Square	F Value	Pr > F
CONC	1	3482.295	3482.295	4.02	0.0505
LITTER	1	12586.348	12586.348	14.53	0.0004
WATER	1	9780.481	9780.481	11.29	0.0015
REP	1	706.725	706.725	0.82	0.3708
CONC*LITTER	1	639.729	639.729	0.74	0.3943
CONC*WATER	1	27.218	27.218	0.03	0.8600
REP(CONC*WATER)	3	3934.318	1311.439	1.51	0.2226
BWT	1	1844753.843	1844753.843	2129.37	<.0001

R-Square	Coeff Var	Root MSE	CWT Mean
0.979334	2.662352	29.43364	1105.550

Appendix4 c: Analysis of variance (ANOVA) for Gizzard weight

Dependent Variable: GIWT

Source	DF	Type III SS	Mean Square	F Value	Pr > F
CONC	1	32.5807978	32.5807978	0.62	0.4340
LITTER	1	71.3674406	71.3674406	1.36	0.2486
WATER	1	41.2553634	41.2553634	0.79	0.3790
REP	1	95.3228342	95.3228342	1.82	0.1834
CONC*LITTER	1	149.9541818	149.9541818	2.86	0.0969
CONC*WATER	1	317.4891075	317.4891075	6.06	0.0174
REP(CONC*WATER)	3	66.4970595	22.1656865	0.42	0.7371
BWT	1	0.5857147	0.5857147	0.01	0.9162

R-Square	Coeff Var	Root MSE	GIWT Mean
0.186449	17.56609	7.235472	41.19000

Appendix 4 d: Analysis of variance (ANOVA) for Liver weight

Dependent Variable: LIWT

Source	DF	Type III SS	Mean Square	F Value	Pr > F
CONC	1	8.6610134	8.6610134	0.23	0.6367
LITTER	1	108.6622065	108.6622065	2.83	0.0986
WATER	1	182.4112886	182.4112886	4.76	0.0340
REP	1	130.5247433	130.5247433	3.41	0.0710
CONC*LITTER	1	6.9252168	6.9252168	0.18	0.6727
CONC*WATER	1	164.4304235	164.4304235	4.29	0.0436
REP(CONC*WATER)	3	253.9942156	84.6647385	2.21	0.0989
BWT	1	2.4351220	2.4351220	0.06	0.8021

R-Square	Coeff Var	Root MSE	LIWT Mean
0.287482	17.90593	6.191272	34.57667

Appendix 5: **Treatment effects for ammonia concentration in litter**

Appendix 5 a: Analysis of variance (ANOVA) for second week of experiment.

Dependent Variable: WK2

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	6	449.4011429	74.9001905	13.99	0.0027
REP	1	0.0000071	0.0000071	0.01	0.9991

R-Square	Coeff Var	Root MSE	WK2 Mean
0.933294	6.417993	2.313732	36.05071

Appendix 5 b: Analysis of variance (ANOVA) for fourth week of experiment.

Dependent Variable: WK4

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	6	813.9505714	135.6584286	11.46	0.0046
REP	1	1.1485786	1.1485786	0.10	0.7659

R-Square	Coeff Var	Root MSE	WK4 Mean
0.919862	10.27749	3.440242	33.47357

Appendix 5c : Analysis of variance (ANOVA) sixth week of experiment.

Dependent Variable: WK6

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	6	3316.474771	552.745795	85.07	<.0001
REP	1	1.137150	1.137150	0.18	0.6902

R-Square	Coeff Var	Root MSE	WK6 Mean
0.988386	7.614825	2.548954	33.47357

Appendix 6: **Treatment combinations effects for ammonia concentration in litter**

Appendix 6 a: Analysis of variance (ANOVA) for second week of experiment.

Dependent Variable: WK2

Source	DF	Type III SS	Mean Square	F Value	Pr > F
CONC	1	72.30031250	72.30031250	18.01	0.0054
LITTER	1	18.06005000	18.06005000	4.50	0.0781
WATER	1	98.35031250	98.35031250	24.51	0.0026
CONC*LITTER	1	2.02005000	2.02005000	0.50	0.5047
CONC*WATER	1	1.99001250	1.99001250	0.50	0.5077

R-Square	Coeff Var	Root MSE	WK2 Mean
0.899449	5.826753	2.003335	34.38167

Appendix 6 b: Analysis of variance (ANOVA) for fourth week of experiment.

Dependent Variable: WK4

Source	DF	Type III SS	Mean Square	F Value	Pr > F
CONC	1	72.24020000	72.24020000	6.76	0.0407
LITTER	1	8.00000000	8.00000000	0.75	0.4203
WATER	1	72.24020000	72.24020000	6.76	0.0407
CONC*LITTER	1	1.03000000	1.03000000	0.50	0.5057
CONC*WATER	1	8.04005000	8.04005000	0.75	0.4192

R-Square	Coeff Var	Root MSE	WK4 Mean
0.727458	10.64909	3.270070	30.70750

Appendix 6 c: Analysis of variance (ANOVA) for sixth week of experiment.

Dependent Variable: WK6

Source	DF	Type III SS	Mean Square	F Value	Pr > F
CONC	1	98.28020000	98.28020000	18.38	0.0052
LITTER	1	8.00000000	8.00000000	1.50	0.2671
WATER	1	50.10005000	50.10005000	9.37	0.0222
CONC*LITTER	1	1.00000000	1.00000000	0.50	0.5048
CONC*WATER	1	2.00000000	2.00000000	0.37	0.5632

R-Square	Coeff Var	Root MSE	WK6 Mean
0.853685	8.448263	2.312290	27.37000