THE EFFECT OF MORINGA SUPPLEMENTATION ON GROWTH AND HEALTH OF INDIGENOUS ZAMBIAN CHICKENS

BY

CHONGWE M ANDREW

SUPERVISORS: DR. DRINAH NYIRENDA, DR JUDITH LUNGU, AND DR J.SIMBAYA

A THESIS SUBMITTED TO THE SCHOOL OF AGRICULTURAL SCIENCES IN PARTIAL FULFILMENT OF THE REQUIREMENTS OF THE AWARD OF DEGREE OF MASTER OF SCIENCE IN ANIMAL NUTRITION

UNIVERSITY OF ZAMBIA

SCHOOL OF AGRICULTURAL SCIENCES

DEPARTMENT OF ANIMAL SCIENCE

LUSAKA

2011

DECLARATION

This research and the results shown herein are a true reflection of the results obtained both at Mazabuka Research Station and at the University of Zambia Field Station. To the best of my knowledge, this study has never been conducted at the aforementioned institutions or any other research institution. This thesis therefore represents solely the findings of the researcher under the guidance of three senior lecturers as my supervisors.

CHONGWE M ANDREW – STUDENT ……

CERTIFICATE OF APPROVAL

'This thesis/dissertation of Chongwe M Andrew has been approved as fulfilling the requirements or partial fulfillment of the requirements for the award of the Degree of Master of Science in Animal Nutrition by the University of Zambia',

…….... ……..

DR. D.B. NYIRENDA – MAIN SUPERVISOR/EXAMINER Signature

…… ……..

DR. K.E.S YAMBAYAMBA - EXAMINER Signature

…… ……..

DR. D.T. DAURA - EXAMINER Signature

ABSTRACT

There are indications that Moringa (*Moringa oleifera*) leaf meal can serve as a feed supplement supplying nutrients and natural antimicrobials/medicines in poultry chicken diets. Two experiments evaluated the effect of Moringa leaf meal as a feed supplement on the growth and health of indigenous Zambian chickens. The first experiment evaluated the response of indigenous Zambian chickens' growth, feed intake and digestibility, and intestinal microbial load to an increasing level of Moringa leaf meal in the diet. Sixty indigenous chickens, blocked by body weight, were randomly assigned to receive isonitrogenous and isocaloric diets containing 0%, 10%, 20% and 30% Moringa leaf meal on weight basis. The experimental design was a 4x3 randomized complete block design. The diet having 0% Moringa leaf meal was the control diet. In both experiments, the chickens had ad libitum access to feed. Individual body weights were taken on a weekly basis while feacal samples were collected fortnightly for digestibility and microbial load assessments, using proximate analysis and plate count respectively. Growth rates were 41%, 49% and 7% higher ($P<0.05$) in indigenous chickens receiving diets with 0%, 10% and 30% Moringa leaf meal, respectively, in comparison with those whose diet had 20% Moringa leaf meal. There were significant differences ($P<0.05$) in faecal bacterial count among the treatments, with diets having 10% Moringa leaf meal having the lowest counts. The second experiment was conducted to evaluate further the effects of Moringa leaf meal on the health of indigenous Zambian chickens at inclusion levels of less than 20% Moringa. This was arrived at based on indications from the first experiment that the indigenous chickens on levels of Moringa below 20% had less mortality. Thus, 60 indigenous Zambian chickens were divided equally between sex and type into 12 groups and assigned at random to three isonitrogenous and isocaloric dietary treatments supplemented with 5%, 10% and 15% Moringa oleifera leaf meal on weight basis. The experimental design was a 3x2x2 factorial design with three Moringa leaf meal levels in the diets, sex (male or female) and chicken type (large or small) as the factors. The duration of the experiment was 8 weeks prior to which the first two weeks were used for the chickens to adapt to the treatments. Blood samples were taken from the wing veins using sterile needles for antibody titre level analysis using the Enzyme Linked Immuno Sorbent Assay method. There were significant

differences between treatment means for antibody titre levels fourteen days after vaccinating against Newcastle disease. The blood antibody titre level for chickens on M10 was over 1000 micro liters whereas those on M5 and M15 had 783 and 876, respectively. There were no differences ($P>0.05$) between treatment means for antibody titre levels before and 7 days after vaccinating against Newcastlle disease. In both scenarios, the M10 treatment had antibody titres at higher but insignificant ($P<0.05$) levels. Moringa leaf meal did affect antibody titre levels before and after vaccinating the flock against Newcastle disease irrespective of chicken type. The results from the two experiments indicate that 10% inclusion rate of Moringa leaf meal in the diet promotes growth and optimum utilization of the natural antimicrobials/medicines.

DEDICATION

I dedicate this thesis to my dear parents, Mr. and Mrs. Chongwe, my wife Michelo and to my children Catherine and Aaron.

ACKNOWLEDGEMENTS

I wish to acknowledge God the almighty, Jesus Christ my Lord and Saviour and the fellowship of the Holy Spirit as the ultimate source of all wisdom, knowledge and providence in this work that has been completed.

My sincere gratitude goes to Dr. Drinah Nyirenda and Dr. Judith Lungu whose encouragement, support and professional advice as my supervisors has sustained me to the end.

The support, encouragement, surprise visits to the study area and advice of Dr. Joseph Simbaya, my other supervisor, are deeply appreciated.

Special thanks go to all the lecturers and support staff of the Department of Animal Science at the University of Zambia who debated the initial project proposal and the parameters to be measured which improved the final proposal and its implementation.

My heartfelt gratitude goes to the Ministry of Livestock and Fisheries Development for facilitating the financial and material support towards this research.

I would like to express my profound appreciation to RUFORUM and ODDESSA for their financial support towards this research.

Last but not the least, I would like to thank all those who contributed in one way or another towards this project.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER ONE

1.0 INTRODUCTION

In Zambia, more than 93% of the population in the rural areas own indigenous Zambian chickens (Haazele et al., 2002). These indigenous Zambian chickens can be sold or bartered to meet essential family needs such as medicines, clothes and school fees (Guèye, 1997; Spradbrow, 1993-94). The major constraint of indigenous Zambian chicken production has been high mortalities due to poor nutrition and diseases attributed to high costs and scarcity of feed ingredients and inadequate health care (Nakamura, 1990).

Recent studies have demonstrated that Moringa (*Moringa oleifera*) leaf meal provides natural antimicrobials/medicines, vitamins, minerals, and proteins (Sarwatt, *et al.,* 2002). Moringa plant is drought resistant and Moringa grows in all types of soils; from acid to alkaline (Duke, 1987), and at altitudes from sea level to 1800 m. It is drought tolerant and will grow even during the 6 months of the dry season in Zambia (May – October). Dry matter (DM) yield is high-15 tons/ha/year. In a study in Nicaragua, fresh leaves were found to contain 23% crude protein (CP) in the DM, 12.3 MJ ME/kg DM and had an in vitro DM digestibility of 79.7% (Becker, 1995). Moringa leaves, petioles and young stems have slightly lower protein content than Gliricidia sepium (26%) and Leucaena leucocephala (25%). However, the CP of Moringa is of better quality for ruminants than the CP of leaves of Gliricidia or Leucaena because of its high content of bypass protein, 47% versus 30% for G. Sepium and 41% for L.Leucaena (Becker, 1995). Moringa is also rich in carotene,

ascorbic acid, iron and in the two amino acids generally deficient in other feeds, i.e. methionine and cystine (Makkar and Becker, 1996).

Yang1 et al. (2006) reported reduced blood triglycerides and enhanced immune response due to increased peripheral and splenocyte T-cell proliferations in rats as a result of Moringa leaf meal inclusion in the diet. Caceres et al. (1991) attributes fast growth in man and livestock to Moringa being able to promote metabolism with its bio-available ingredients and their ability to increase the natural defenses of the body.

1.1 Problem Statement

Although knowledge has been acquired with regard to the nutrient contents and natural antimicrobials/medicines properties of Moringa through numerous studies, its potential for use as a supplement in the diets of indigenous Zambian chickens is yet to be explored. Therefore, its effects on the growth and health of indigenous chickens are unknown.

1.2 Justification

There is need to determine the extent to which Moringa leaf meal can be utilized by indigenous Zambian chickens and the effects it would have on the health of the chickens when used as a supplement. The accepted levels would then be adopted to provide a cheap and readily available source of nutrients and antimicrobes in the diets of the indigenous chickens. This would greatly help rural populations with indigenous Zambian chickens to improve the nutrition of their chickens, leading to an increased poultry population and thus have a source of food and income generation through the sale of the chickens. Being a drought resistant plant, Moringa can be grown and

utilized by both humans and poultry considering that this nutrition and antimicrobials/medicine will be available when other food sources may be scarce.

1.3 Overall Goal of the Research

To contribute to rural wealth creation through enhanced indigenous Zambian chicken production by application of unconventional feed supplements.

1.4 Main Objective

The main objective of this research was to assess the effectiveness of Moringa as a feed supplement on the productivity of indigenous Zambian chickens.

1.5 Specific Objectives

1) To evaluate and compare indigenous Zambian chicken's feed intake, growth rate, digestibility, and adult weight attainment with varying supplement levels of Moringa leaf meal.

2) To study antimicrobial effects of varying supplement levels of Moringa on performance of the indigenous chickens.

3) To study the antibody titre to Newcastle disease under Moringa leaf meal supplementation.

1.6 Research Hypothesis

Growth and health of indigenous Zambian chickens can be improved through the use of Moringa leaf meal supplementation.

CHAPTER TWO

2.0 LITERATURE REVIEW

Available literature has revealed that endeavors to improve livestock production and productivity have been made through improvement of nutrition by use of Moringa leaf meal. Fahey (2005) reported that Moringa was coming to the forefront as a result of scientific evidence that it is an important source of nutrients, natural antimicrobials/medicines and other naturally occurring plant chemicals (phytochemicals) such as alkaloids, glucosinolates and isothiocyanates that provide anti-hypertensive, diuretic and cholesterol lowering activities.

In a study by Kakengi et al. (2007) it was revealed that Moringa could be used as a source of plant protein since it was highly accepted even at high inclusion levels in commercial layers diets. The study further revealed that the egg laying percentage, egg weight and egg mass production remained within the recommended levels even when Moringa leaf meal was given as a sole plant source, promoting the highest performance in egg production in comparison with other leaf meals already studied. However, it is not known whether there was any effect on the growth and health performance of the layers by the different levels of Moringa leaf meal included in the diets.

In a trial with goats, the inclusion rate of 9%, 27% and 36% Moringa leaf meal in the ration resulted in basal feed dry matter intakes of 251, 335 and 311 g/day, respectively. However, incorporation of different levels of Moringa leaf meal did not affect the body weight (BW) gain - 16.1, 15.0 and 13.6 g/day, respectively (Sarwatt et al., 2002). Goats supplemented with 20% and 50% Moringa leaves had DMI of 50.9 and 51 g/kg$W^{0.75}$ per day, which were similar to the intake of goats that were not supplemented.

The antibacterial principle found in Moringa leaf meal known as pterygospermin, a compound that readily dissociates to benzyl isothiocyanate pterygosperma kills bacteria. It does so by blocking the enzymes cysteine proteinase and alcohol dehydrogenase and thus blocks bacteria metabolism and survival (Yang1 et.al 2006). The antibacterial principle inhibits protein synthesis by binding to the 23S rRNA molecule (in the 50S subunit) of the bacterial ribosome blocking the exit of the growing peptide chain of sensitive microorganisms. Certain resistant microorganisms with mutational changes in components of this subunit of the ribosome fail to bind the drug. The association between erythromycin and the ribosome is reversible and takes place only when the 50 S subunit is free from tRNA molecules bearing nascent peptide chains. Gram-positive bacteria accumulate about 100 times more erythromycin than do gram-negative microorganisms (Ivan et al., 1993). Pterygospermin also inhibits bacterial protein synthesis by blocking the attachment of the transfer RNA-amino acid to the ribosome.

In a study by Yang1 et al. (2006), the immune modulation of dried Moringa powder in diets for human use and livestock production intervention with a diet containing 5% Moringa powder was investigated. Using a rat model and compared to a 5% common cabbage diet, and a nutrient-sufficient diet without vegetable. Results indicated that the Moringa diet lightly reduced blood triglycerides and enhanced immune response due to increased peripheral and splenocyte T-cell proliferations. The implication is that the consumption of Moringa may increase immune response of subjects. The same study revealed that consumption of nutrient and phytochemical-rich vegetables like Moringa, led to a better immune response compared to consumption of vegetables that are rich in fiber but low in nutrient or phytochemical content like common cabbage. The same group of scientists (Yang1 et al., 2006) also studied the effects of dehydrated leaves of Moringa in the diets of 21 broilers where 5 treatments (diet without Moringa and diets containing 0.5%, 1%, 2% and 3% dried leaves) were used for three weeks. Results indicated that Moringa diets significantly: (1) enhanced duodenum traits by having an increased number of ileum microflora that lead to an increased absorption surface area; (2) increased concentrations of total globulin, γ-globulin and lymphocyte ratio, antibody titer levels with comparison to the standard (sheep erythrocytes), and delayed type hypersensitivity; (3) reduced *E. coli* and increased *Lactobacillus* counts in the ileum.

Rocha and Mendieta (1998) conducted a study in which they fed dairy cows with *Hyparrhenia rufa* grass and sorghum straw supplemented with different levels of Moringa leaves. The Moringa leaves were readily accepted by the animals and did not seem to have any toxic effect or contain any factors limiting intake. Supplementation with Moringa leaves at a level of 0.3% of body weight resulted in a milk yield of 5.7

kg cow^{-1} day^{-1}, and this was 13% higher than for the control treatment, which was grazing only. Sarwatt et al. (2004) found that when cotton seed cake was substituted by Moringa leaf meal at levels of 10, 20 or 30% of DM, milk yield was significantly increased by 1.4, 0.9 and 0.8 kg cow day^{-1} respectively. However, there were no effects of substituting cotton seed cake with Moringa leaf meal on total solids, fat and CP content of the milk.

The use of sub-therapeutic levels of antibiotics in poultry feed has been found to improve performance and lower morbidity. Moringa roots have antibacterial activity (Rao et al., 1998) and are reported to be rich in antimicrobial agents. These are reported to contain an active antibiotic principle, pterygospermin, which has powerful anti-bacterial and anti-fungal effects (Ruckmani et al., 1998). A similar compound is found to be responsible for the antibacterial and antifungicidal effects of its flowers (Das et al., 1957). The root extract also possesses antimicrobial activity attributed to the presence of 4-alpha-L-rhamnosyloxy benzyl isothiocyanate (Eilert et al., 1981). The aglycone of deoxy-niazimicine (N-benzyl, S-ethyl thioformate) isolated from the chloroform fraction of an ethanol extract of the root bark was found to be responsible for the antibacterial and antifungal activities (Nikkon et al., 2003). The bark extract has been shown to possess antifungal activity (Bhatnagar et al., 1961), while the juice from the stem bark showed antibacterial effect against Staphylococcus aureus (Mehta et al., 2003). The fresh leaf juice has been found to inhibit the growth of microorganisms (Pseudomonas aeruginosa and Staphylococcus aureus), pathogenic to man (Caceres et al., 1991).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 EXPERIMENT ONE

3.1.1 Location

The first experiment was carried out at Mazabuka Research Institute and the duration of the experiment was 12 weeks. Figures 1a and 1b show some of the pens in which indigenous Zambian chickens were kept during the experiment.

3.1.2 Experimental Design

A total of sixty indigenous Zambian chickens, of mixed type, each weighing 110 grams on average, were purchased from a local farmer in Kasisi, Lusaka. The indigenous chickens were hatched from a home-made incubator and were two weeks old at the time they were bought. After an adaptation period of two weeks, the chicks were equally divided into three groups according to body weight to form blocks. The three groups were further divided into four groups of five chicks each. These groups were assigned at random to four dietary treatments. At the start of the experiment, the indigenous chickens were four weeks of age and weighed 308 grams on average. The dietary treatments had 0%, 10%, 20% or 30% Moringa leaf meal on weight basis. The diets were isonitrogenous and isocaloric. The experimental design was a 4x3 Randomized Complete Block Design. In this experiment, the indigenous chicken was the experimental unit, the four formulated diets were the treatments, and the blocking factor was body weight. The experiment was run for twelve weeks. Prior to the

commencement of the experiment, a one week period was used to allow the chickens to adapt to the diets.

Figure 1a

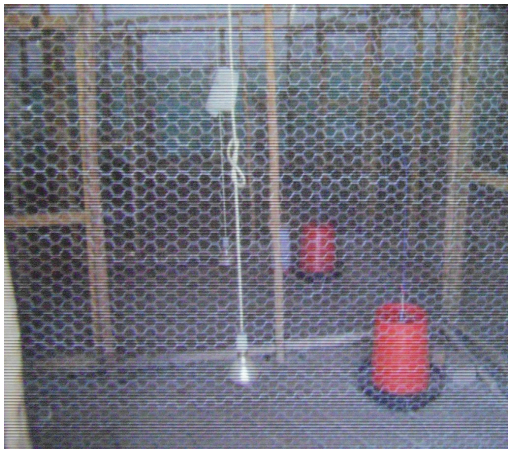

Figure 1b

Figures 1a and 1b: Shows some of the indigenous chickens and pens used in experiment one conducted in Mazabuka.

3.1.3 Management of the Chickens

The poultry house where the experiment was run was thoroughly cleaned and disinfected. It was then demarcated into twelve pens by using chicken wire and wooden poles (Figure 1b). The chickens were kept on the floor with 5 birds per pen. They had access to feed and water throughout the day. Fresh water was supplied every day.

3.1.4 Experimental Diets

Four isonitrogenous and isocaloric experimental diets were formulated to obtain diets having 0%, 10%, 20% and 30% Moringa oleifera leaf meal on weight basis designated as M0, M10, M20 and M30, respectively (Table 1).

3.1.5 Source of Moringa oleifera Leaf Meal

Moringa leaves were harvested from an orchard within Lusaka (Makeni area). The cut branches were spread out on a concrete floor and allowed to dry for a period of 4-5 days under shady conditions after which the leaves were separated from the twigs. The leaves were then pounded to produce the leaf meal by using a mortar and pestle.

3.1.6 Biochemical Analysis of feed

Samples of Moringa oleifera leaves, Soya beans and number 3 maize meal were subjected to proximate analysis according to the Association of Official Analytical Chemists (AOAC, 2000) methods. All the analyses were done in triplicate.

3.1.7 Feed Intake and Bodyweights

The bodyweights were taken as absolute weights of the individual birds and this was done with the use of a sensitive balance (0.005g) having capacity of three decimal places. The birds were weighed once every week for twelve weeks.

Feed intake was determined by weighing the amount of feed given at the beginning of the week and the remaining feed at the end of the week. The difference in amount was taken as the amount of feed consumed. The value was then divided by the total number of chickens housed in the pen to come up with the amount of feed consumed per bird. Whenever there was mortality, the feed was weighed to determine the amount of feed consumed by the chickens before the mortality and after.

Table 1: Composition of Experimental Diets for experiment one

	Diets			
Ingredients [Kgs]	M0 (Control)	M10	M20	M30
Moringa leaf meal	0.00	10.00	20.00	30.00
Maize (Number 3 Meal)	82.38	70.00	60.30	50.00
Soyabean meal	13.50	11.00	5.80	2.00
Dicalcium Phosphate	2.43	2.43	2.43	2.43
Methionine	0.92	0.92	0.92	0.92
Salt	0.03	0.03	0.03	0.03
Phosphorus	0.74	0.74	0.74	0.74
Cottonseed hulls	-	4.88	9.78	13.88
Chemical analysis (%)				
DM	91.95	91.60	91.36	91.10
ME (MJ/Kg)	14.58	14.74	13.75	14.13
CP	19.92	19.30	19.77	19.70
CF	3.60	3.40	3.80	4.40
EE	11.99	12.52	9.91	11.98
Ash	9.09	8.34	11.25	11.29
Ca	1.74	1.22	1.80	1.34
P	1.66	1.05	0.90	1.12

MOLM = *Moringa oleifera* leaf meal, DM = Dry Matter, ME = Metabolisable Energy, CP = Crude Protein, CF = Crude Fibre, EE = Ether Extract, Ca = Calcium, P = Phosphorus.

Feacal samples were collected fortnightly for microbial and digestibility tests. Fresh samples were collected over a period of 48 hours. This was done very early in the morning and the samples were stored in sterile test tubes below $0^{o}C$ to prevent

bacterial degradation. Feed intake was recorded and used in digestibility calculations. Four samples were collected per treatment giving a total of twelve samples per treatment since each treatment was replicated thrice. The samples were taken to the laboratory at the University of Zambia's Animal Science and Food Science Bacteriology laboratory for digestibility and microbial analysis respectively. Proximate analysis of the nutrients in the feacal samples were done by using the Association of Official Analytical Chemists (AOAC, 2000) methods.

3.1.8 Feacal Analysis

For apparent digestibility tests, the feaces were dried and finely ground before analysis. The nutrients analysed included Metabolisable Energy, ether extract, phosphorus, calcium and crude protein. These were done in triplicate using the Association of Official Analytical Chemists (AOAC, 2000) methods. Microbial analysis of the feacal samples was done by using the microbial plate count method after soaking the feacal samples in Dilute Phosphate Buffered Saline salt (DPBS).

3.1.9 Statistical Analysis

Production parameters measured during the experiment included live-weights, weight gain, cumulative weight gain, feed consumption and feed conversion ratio. The data collected for each parameter were captured using Microsoft windows excel software. The data were then imported into the SAS software and statistically analyzed for each parameter measured. Values were considered significant at $P<0.05$. T-test analysis was done for final mean live-weights. Regression analysis was done between the percentage of *Moringa oleifera* leaf meal and intestinal microbial load. Treatment means were separated by Duncan multiple range test - least square means (Lsd). The

data for production (liveweight, weight gain, cumulative weight gain, feed consumption and feed conversion ratio) were analysed using Model 1.

Model 1: $Y_{ijk} = U + A_i + T_j + (AT)_{ij} + E_{ijk.}$

Model 1: $Y_{ijk} = U + A_i + T_j + (AT)_{ij} + E_{ijk}$

Where:

U = overall mean

Y_{ij} = Observations of k^{ith} bird assigned to i^{th} level of Moringa leaf meal taken at j^{th} weeks of age

A_I = Effect associated with the i^{th} level of Moringa leaf meal

T_j = Effect associated with j^{th} weeks of age

$(AT)_{ij}$ = Interaction between Moringa leaf meal levels and age (weeks)

E_{ij} = Random error

3.2 EXPERIMENT TWO

3.2.1 Location and Experimental Design

Experiment two was conducted at the University of Zambia (Great East Road campus) Field Station livestock unit. The aim was to evaluate further the effects of Moringa leaf meal on the health of indigenous Zambian chickens at inclusion levels of less than 20%. This was arrived at based on indications from experiment 1 that chickens on levels below 20% had less mortality. The experimental design was a 3x2x2 factorial design with three Moringa leaf meal levels in the diets (5%, 10% and 15%), sex (male or female) and chicken type (large or dwarf). The diet having 10%

Moringa leaf meal gave the best growth and health performance during experiment 1 and hence was used as a reference diet in experiment 2. Sixty indigenous chicks were divided equally between sex and body size. The chicks were sourced from the same farmer as in experiment 1. Unlike in experiment 1, sex was included as a factor because of the fact that male chickens tend to consume more feed and have higher weight gains than female chickens of the same age. Hence there was need to determine how Moringa oleifera leaf meal influenced the health of the indigenous chickens according to how much feed was consumed according to sex. Type was also used to categorize the chickens according to body size. This is because there are three types and includes the large, normal and dwarf. Due to financial constraints, it was opted to use the large type and small or dwarf type only. Figure 2 shows one of the four cages used in experiment 2.

3.2.2 Management of the Chickens and Source of Moringa Leaf Meal

The management of chickens and source of Moringa were the same as in experiment 1.

Figure 2: One of the four cages used in experiment two at the University of Zambia's field station.

3.2.3 Experimental Diets

Three isonitrogenous and isocaloric experimental diets were formulated to obtain diets having 5%, 10% and 15% Moringa leaf meal on weight basis designated as M5, M10 and M15, respectively (Table 2).

Table 2: Composition of Experimental Diets for experiment two

	Diets		
Ingredients [Kgs]	M5	M10	M15
Moringa leaf meal	5.00	10.00	15.00
Maize (Number 3 Meal)	77.3	70.00	60.30
Soyabeans	13.00	11.00	5.80
Dicalcium Phosphate	2.43	2.43	2.43
Methionine	0.92	0.92	0.92
Salt	0.03	0.03	0.03
Phosphorus	0.74	0.74	0.74
Cottonseed hulls	0.58	4.88	14.78
Chemical analysis (%)			
DM	90.60	93.36	88.10
ME (MJ/Kg)	14.02	14.95	14.03
CP	19.99	19.64	19.23
CF	4.85	6.79	2.56
EE	13.20	10.78	12.05
Ash	13.62	12.50	12.78
Ca	1.72	1.55	1.47
P	1.99	1.79	1.44

MOLM = *Moringa oleifera* leaf meal, DM = Dry Matter, ME = Metabolisable Energy, CP = Crude Protein, CF = Crude Fibre, EE = Ether Extract, Ca = Calcium, P = Phosphorus.

3.2.4 Biochemical Analysis of Diets

Samples of Moringa oleifera leaves, soyabeans and number 3 maize meal were subjected to proximate analysis according to the Association of Official Analytical Chemists (AOAC, 2000) methods. All the analyses were done in triplicate.

3.2.5 Blood collection and Analysis

Blood samples were obtained from three chickens per replicate making a total of 9 per treatment. This was done by inserting a 19 mm gauge sterile needle into the wing vein of the birds and extracting 2 milliliters of blood. The blood collected was placed into sterile test tubes containing Ethylene Diamine Tetra Acetic Acid (EDTA). The blood samples were shaken in order to thoroughly mix with the EDTA to prevent coagulation. The samples were then taken to the laboratory for antibody titre level analysis using the Enzyme Linked Immuno Sorbent Assey.

3.2.6 Statistical Analysis

Blood sample results from the laboratory were subjected to Analysis of variance (ANOVA) according to Snedecor and Cochran (1992) using general linear model (GLM) procedures of Statistical Analysis System (SAS) Inc, (1998). Values were considered significant at $P<0.05$. Treatment means were separated by Duncans' multiple range test and least square means (Lsd).

Model 2: $Y_{ijk} = U + A_i + T_j + (AT)_{ij} + b\,(x_{ijk}-x) + E_{ijk}$

Where:

U = Overall mean

Y_{ijk} = Observations of k^{th} bird assigned to i^{th} level of Moringa oleifera leaf meal taken at j^{th} weeks of age

A_I = Effect associated with the i^{th} level of Moringa oleifera leaf meal

T_j = Effect associated with j^{th} weeks of age

$(AT)_{ij}$ = Effect associated with interaction between treatments and age (weeks)

Xijk = Initial body weight of an individual

X = Overall mean for initial body weight

b = Regression coefficient of Y_{ijk} on x_{ijk}

E_{ij} = Random error

CHAPTER FOUR

4.0 RESULTS

4.1 Experiment One

4.1.1 Nutrient Analysis of Moringa, Soyabean and Maize Meals.

The results for the nutrient analysis done on Moringa leaf meal, soyabean meal and number 3 maize meal at the University of Zambia's Animal Science Laboratory are presented in Table 3 below.

Table 3: Proximate Composition of Moringa leaf meal, Soyabean meal and Maize meal used in experiment one

Nutrients/Parameters	**Moringa**	**Soyabeans**	**Maize**
DM (%)	90.16	90.00	88.00
ME (Kcal/Kg)	3271	2982	3470
CP (%)	26.61	34.00	8.50
CF (%)	3.60	4.30	1.50
EE (%)	10.00	-	-
Ash (%)	10.79	-	-
Ca (%)	3.90	0.07	0.12
P (%)	0.038	0.06	0.11

DM = Dry Matter, ME = Metabolisable Energy, CP = Crude Protein, CF = Crude Fibre, EE = Ether Extract, Ca = Calcium, P = Phosphorus

4.1.2 Effect of Moringa Supplementation on Growth Performance.

At the end of the experiment, there were significant differences ($P<0.05$) between treatments M0, M10 and M30 with regard to final body weights. Treatment M10 had a value of 972.43 grams and was significantly different from M0 and M30 that had values of 890.67 grams and 640.03 grams, respectively. The weekly body weights are presented in (Appendix A1). Trends in the growth patterns of the indigenous Zambian chickens are illustrated in Figure 1. At the beginning of the experiment (week 0), the average body weight was 308 grams. The weights for the chickens did not differ statistically ($P<0.05$) in week one, whereas they differed from week two onwards. Chickens on M10 had the highest growth pattern between week three and eight, and slightly less than those on M0 from week nine to week twelve. The chickens on M0 had a consistent growth pattern throughout most of the study period. Chickens on M30 had a slump in growth in the fourth week and the growth pattern was lower than that for the chickens on M0 and M10. The chickens on M20 had the lowest growth pattern with more growth variations until after week ten.

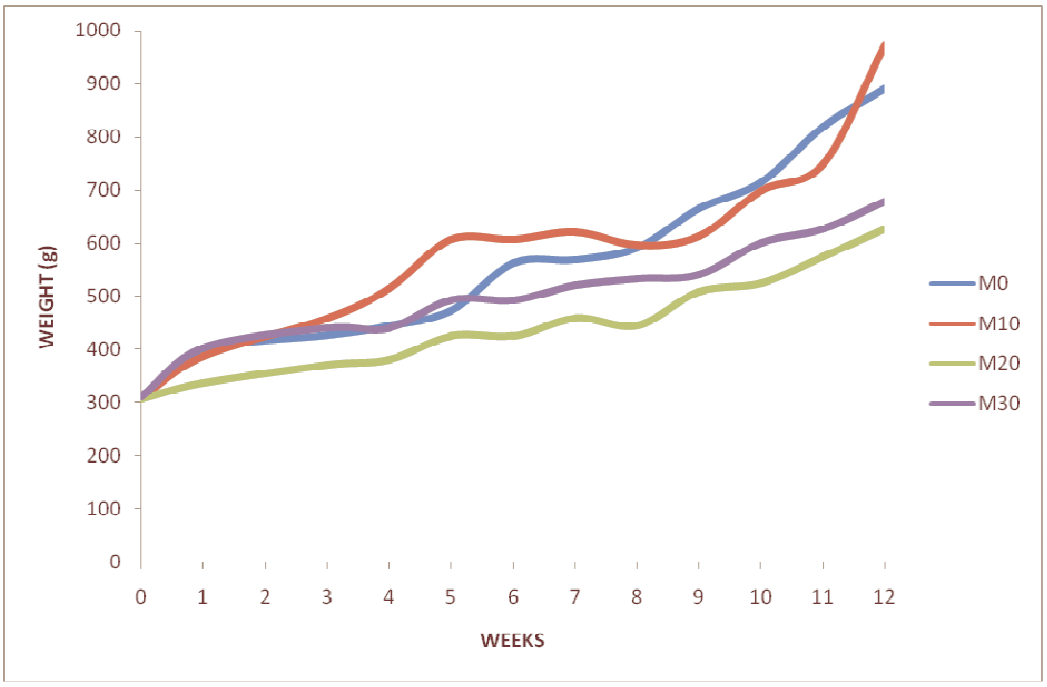

Figure 3: Trends for weekly live-weights

M0 = control diet without Moringa (0%); M10 = treatment diet with 10% Moringa; M20 = treatment diet containing 20% Moringa; M30 = treatment diet having 30% Moringa.

In experiment one, there were significant differences (P<0.05) between treatments M0 and M10 as shown by the T-test results in Table 4 below.

Table 4: T-Test analysis for final mean live-weights

TREAT	N	Mean
M10	14	972.43[a]
M30	10	646.78[b]
M20	8	640.03[b]
M0	6	890.67[a]

Means with different supescripts are significantly different at P< 0.05
Least Significant Difference= 166.45, STD DEV = 169.70
N = number of chickens

4.1.3 Effect of Moringa oleifera leaf meal on health performance of indigenous Zambian chickens.

The mortalities that occurred in experiment one were attributed to an outbreak of fowl pox, endemic in the area where the study was conducted. Out of a total mortality of 22 chickens, 9 were from the control diet {without Moringa leaf meal (0%)}, 7 were from the diet with 20% Moringa leaf meal. Those on 10% and 30% Moringa leaf meal supplementation had 1 and 5 mortalities, respectively.

4.1.4 Effect of Moringa oleifera leaf meal on feed intake of indigenous Zambian chickens.

The results for the mean feed consumption from week one to twelve are shown in appendix A2. The results obtained revealed that there were no significant differences ($P<0.05$) in the total DM intake by village chickens on the different treatments but the trend was that those on M0 tended to eat more (398.5 g/day) followed by M10 (391.3 g/day) and M20 (345.4 g/day), while M30 chickens had the lowest intake (173.8 g/day).

4.1.5 Effect of Moringa oleifera leaf meal on digestibility of nutrients.

The apparent digestibility for Metabolisable energy for M0 and M10 were significantly ($P<0.05$) higher than those for M20 and M30. These were 89.56%, 85.15%, 76% and 80%, respectively. For crude protein, the apparent digestibilities for M0 and M10 were significantly ($P<0.05$) higher than those for M20 and M30. The apparent digestibilities for phosphorus for treatments M10 and M30 were significantly ($P<0.05$) higher than those for M0 and M20. Figures 4 and 5 below show the apparent digestibility results obtained during the study.

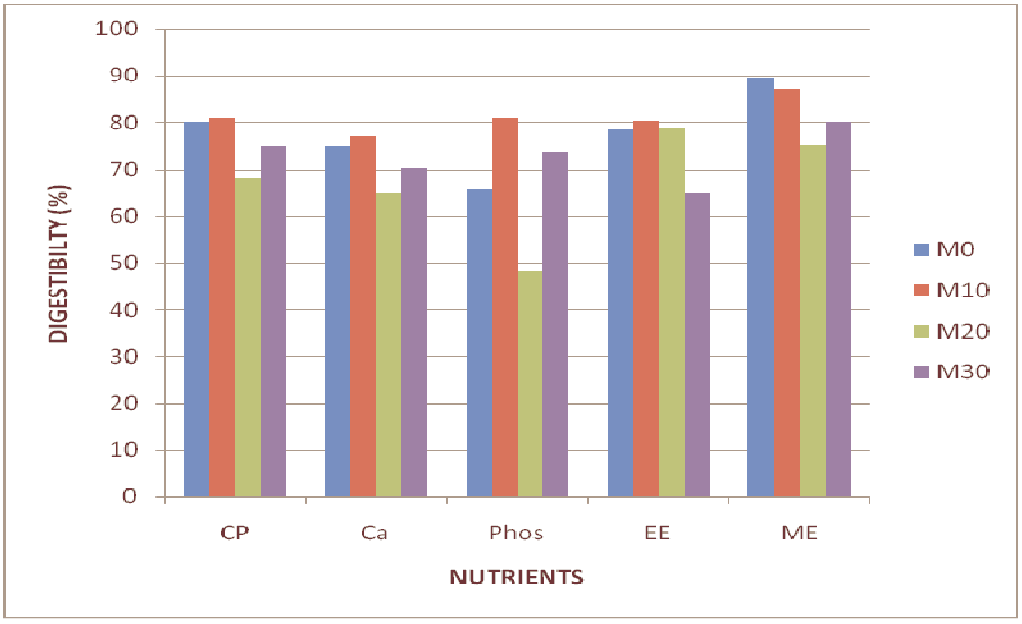

Figure 4: Results for apparent digestibility of the treatment diets.

M0 = control diet without Moringa (0%); M10 = treatment diet with 10% Moringa; M20 = treatment diet containing 20% Moringa; M30 = treatment diet having 30% Moringa. (CP = crude protein, Ca = calcium, Phos = Phosphorus, EE = ether extract, ME =Metabolisable energy).

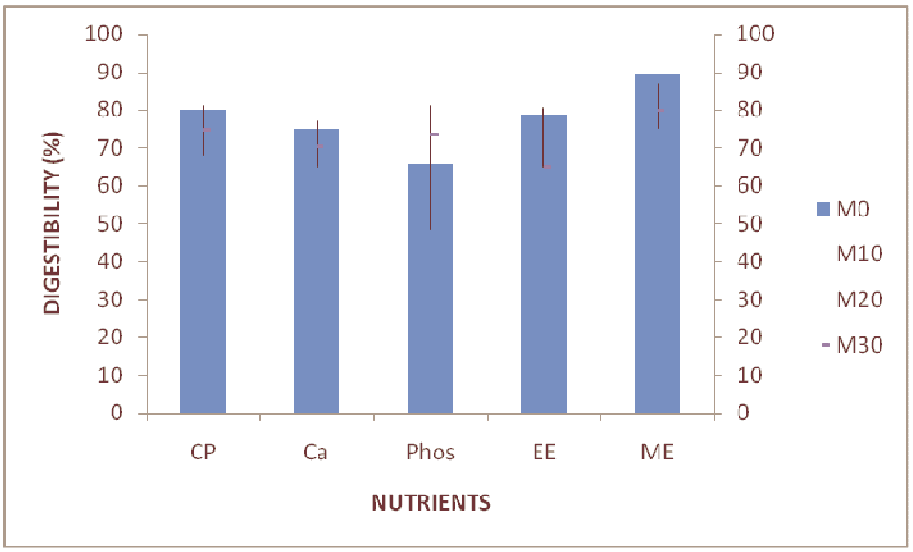

Figure 5: Results showing the highest and lowest digestibility percentages.

M0 = control diet without Moringa (0%); M30 = treatment diet having 30% Moringa. (CP = crude protein, Ca = calcium, Phos = Phosphorus, EE = ether extract, ME =Metabolisable energy).

4.1.6 Effect of Moringa oleifera leaf meal on intestinal microbial load of indigenous Zambian chickens.

Increased levels of Moringa leaf meal reduced the microbial colonies of feacal samples as shown Table 5. The table represents the mean values of the bacterial colonies per gram of feacal samples from the indigenous chickens on the four treatment diets. These were obtained on a weekly basis. The highest count of bacteria was found on M0, followed by those on M10. The results indicate that the chickens on M20 and M30 treatments had lower counts. The following bacteria were found in the feacal samples: *Escherichia coli, Staphylococcus* spp, Bacillus spp, *Corynebacteria* spp, *Streptococcus* spp.

Table 5: Effects of Moringa oleifera leaf meal on microbial colonies per gram of feacal sample

	Week				
	7	8	9	11	
Treatment			**Microbial Load**		**TOTAL**
M0 (Control)	8×10^5	4.5×10^5	1.4×10^7	6.4×10^6	**2.165×10^7**
M10	5.1×10^4	2.6×10^4	1.4×10^7	1.5×10^6	**1.557×10^7**
M20	3.4×10^5	9×10^6	1.7×10^6	1.3×10^5	**9.166×10^6**
M30	6.3×10^4	8×10^5	1.11×10^6	2×10^4	**1.99×10^6**

M0 = control diet without Moringa (0%); M10 = treatment diet with 10% Moringa; M20 = treatment diet containing 20% Moringa; M30 = treatment diet having 30% Moringa.

4.1.7 Regression Analysis between amount of *Moringa oleifera* in the feed and the intestinal microbial load.

Regression analysis done between the amount of Moringa in the feed and the intestinal microbial load found in the feacal samples did not show any statistical differences ($P<0.05$). A regression coefficient (R^2) of 0.3282 was obtained while correlation coefficient (r) value of 0.5 was obtained, showing a negative relationship. The chickens on the diet without Moringa leaf meal had the highest microbial load, followed by those on M10. The least microbial loads were found in M20 and M30. Figure 6 below shows a slight non significant negative relationship with a computed r value of 0.5 indicating that an increasing amount of Moringa leaf meal reduced the intestinal microbial load.

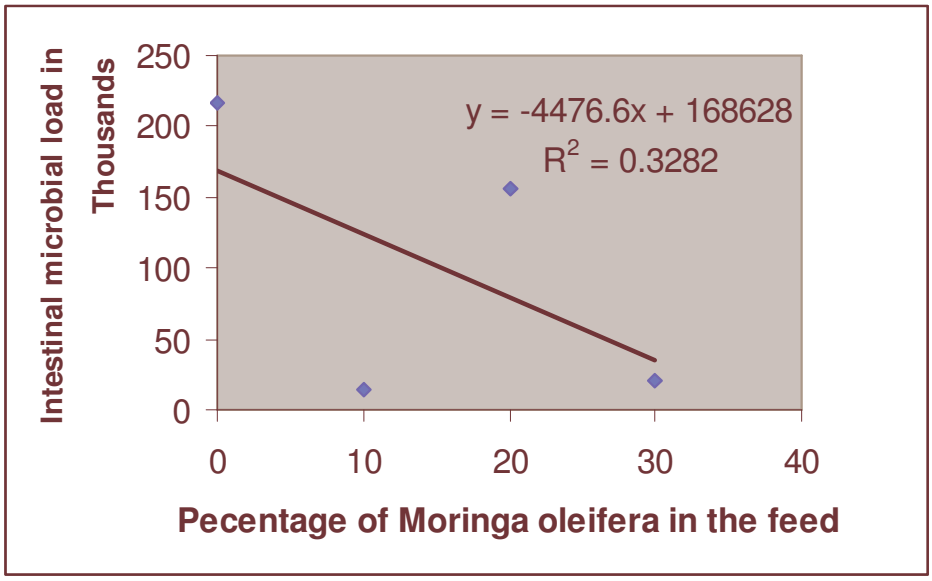

Figure 6: Results for regression analysis between the percentage of Moringa oleifera leaf meal and intestinal microbial load.

4.2 Experiment Two

4.2.1 Nutrient analysis of Moringa oleifera leaf meal, soyabeans and number 3 maize meals.

The results for the nutrient analysis done on Moringa leaf meal, soyabean meal and number 3 maize meal at the University of Zambia's Animal Science Laboratory are presented in Table 6.

Table 6: Proximate Composition of Moringa oleifera leaves, Soya beans and Maize meal used in experiment two

Nutrients/Parameters	**Moringa**	**Soyabean**	**Maize**
DM (%)	90.16	90.00	88.00
ME (Kcal/Kg)	3308	2782	3405
CP (%)	27.61	36.50	9.50
CF (%)	3.87	3.58	1.93
EE (%)	8.00	-	-
Ash (%)	11.20	-	-
Ca (%)	3.90	0.07	0.12

4.2.2 Effect of Moringa oleifera leaf meal on feed intake of indigenous Zambian chickens.

There were significant differences in DM intake of chickens on the different treatments ($P<0.05$). Figure 7 shows the total feed consumed according to the chicken

size/sex, while the Duncan's multiple range test for feed consumption according to treatments and chicken size are shown in table 7.

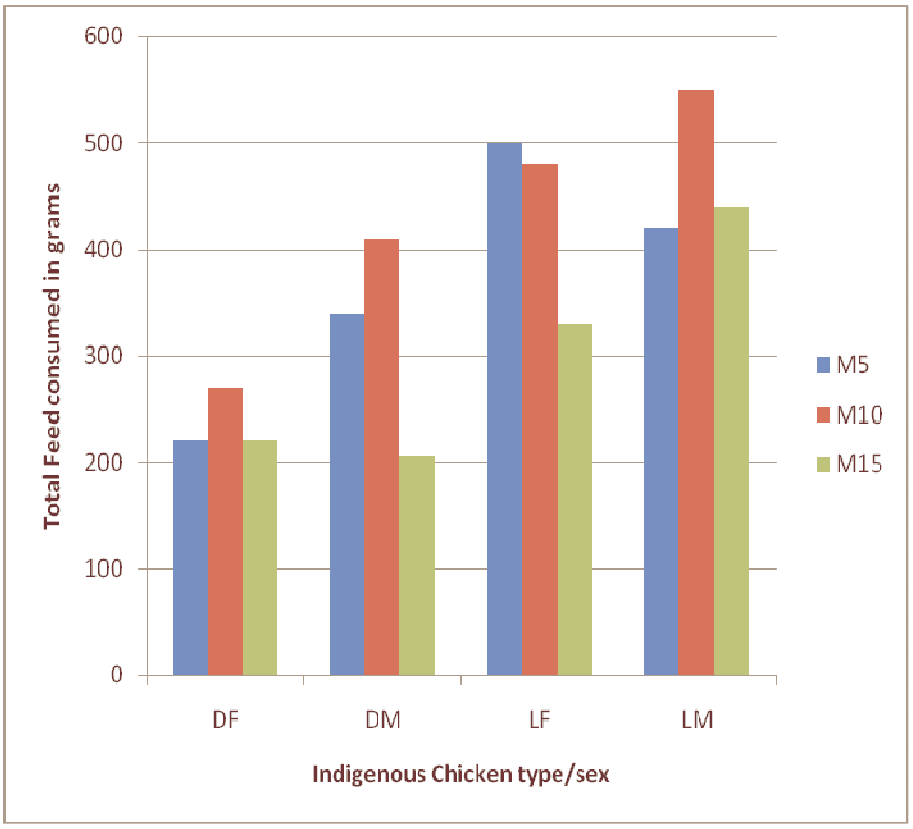

Figure 7: Feed intake of indigenous Zambian chickens.

DF = Dwarf type and whose sex is Female; DM = Dwarf type and whose sex is Male; LF = large type and whose sex is Female; LM = large type and whose sex is Male.

Table 7: Mean Feed Consumption for experiment two

Treatment	Mean feed consumption (g/day)
M10	71.05[a]
M5	57.42[b]
M15	55.76[b]
Size/Sex	**Mean**
Large Female	72.22[a]
Large Male	69.61[a]
Dwarf Male	57.11[b]
Dwarf Female	42.03[c]

Means with different letters are significantly different (P< 0.05).

Treatment LSD = 7.21

Sex/Size LSD = 8.67

4.2.3 Effect of Moringa oleifera leaf meal on health performance of indigenous Zambian chickens.

In experiment two the mortalities that occurred came about after challenging the indigenous chickens with the Newcastle Virus. Out of a total mortality of 17 chickens, 2 (12%) were from the control diet, 0 from the diet with 10% Moringa leaf meal, 9 (53%) were from the diet with 5% Moringa leaf meal. Those on 15% Moringa leaf meal supplementation had 6 (35%) mortalities.

4.2.4 Effect of Moringa oleifera leaf meal on blood antibody levels of indigenous Zambian chicken type/sex.

Figure 8 shows the results for the antibody levels before the birds were vaccinated against Newcastle disease. Although the test is negative at values less than 1000 micro litres, the highest values were found in birds that were on M10 apart from the large female type. Statistically, there were no differences ($P<0.05$) among the blood antibody levels.

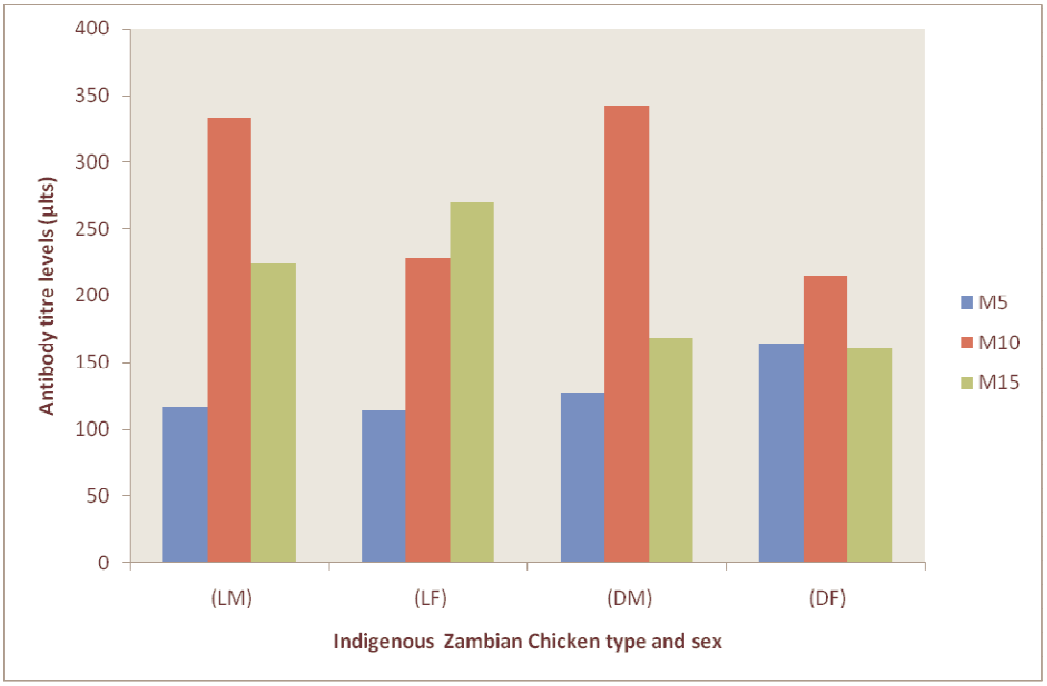

Figure 8: First antibody titre levels.

DF = Dwarf type and whose sex is Female; DM = Dwarf type and whose sex is Male; LF = large type and whose sex is Female; LM = large type and whose sex is Male.

Figure 9 shows the results for the antibody levels 7 days after the birds were vaccinated against Newcastle disease. There were no significant differences ($P<0.05$) between treatment means for the antibody titre levels 7 days after vaccination. Moringa leaf meal induced higher antibody production in males than in females at 10%.

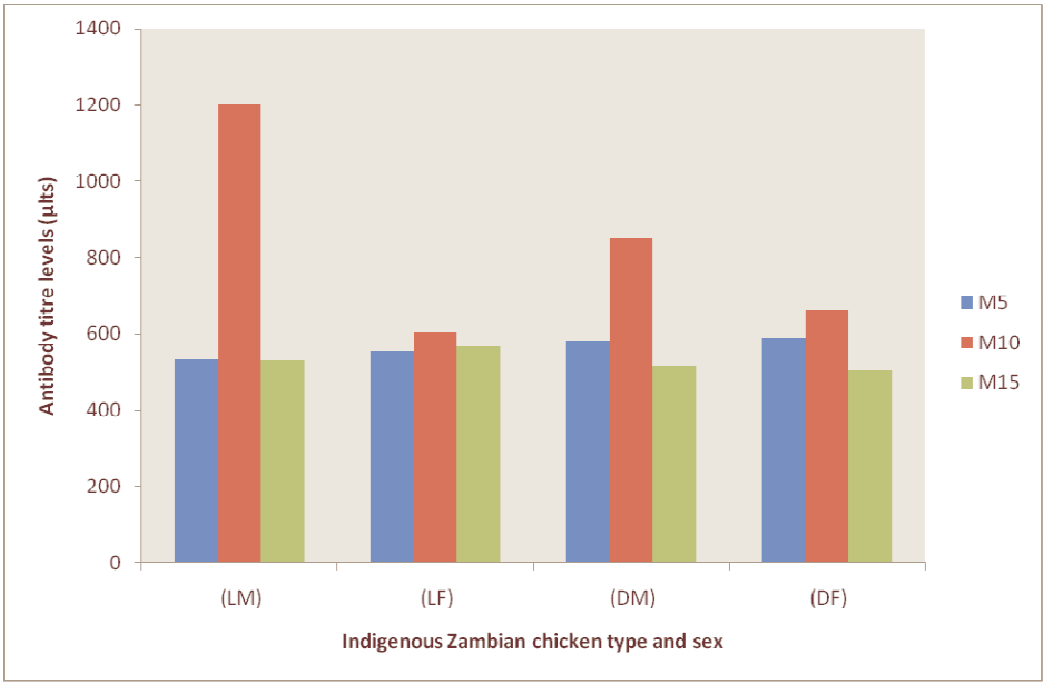

Figure 9: Second antibody titre level analysis

DF = Dwarf type and whose sex is Female; DM = Dwarf type and whose sex is Male; LF = large type and whose sex is Female; LM = large type and whose sex is Male.

Antibody titre levels 14 days after the birds were vaccinated against Newcastle disease are presented in figure 10 below. The results from M10 differed significantly ($P<0.05$) from M5 and M15. The mean value for M10 was 1029 micro litres which was higher than the values of 783 and 876 micro litres for treatments M5 and M15 respectively. The actual numerical data obtained in micro litres are presented in the tables in appendices B1, B2, and B3.

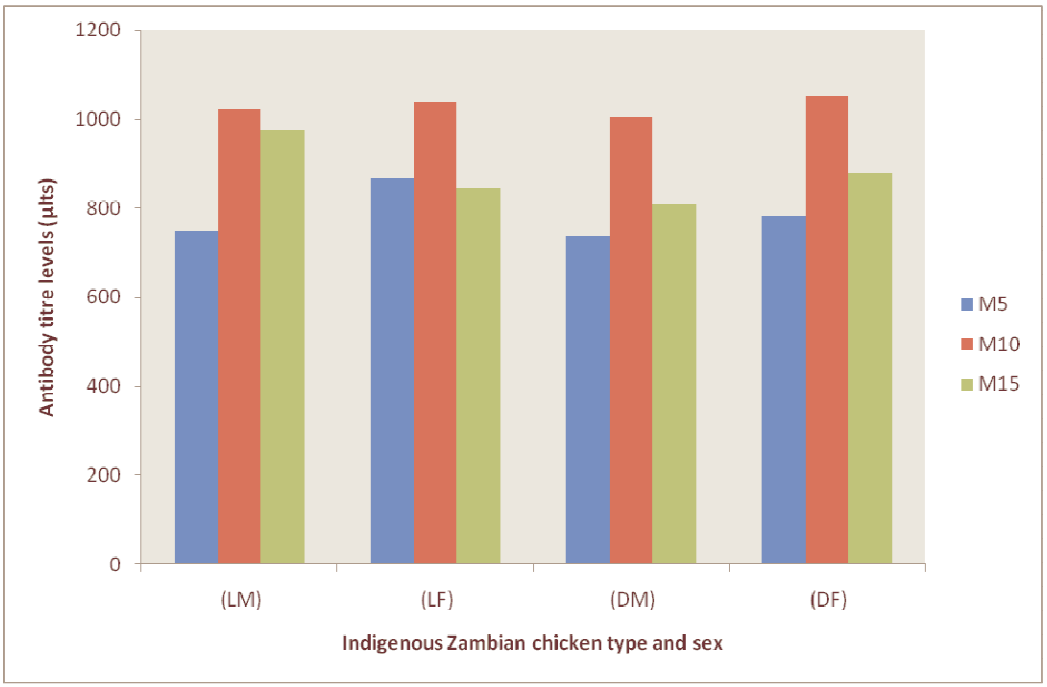

Figure 10: Third antibody titre levels analysis

DF = Dwarf type and whose sex is Female; DM = Dwarf type and whose sex is Male; LF = large type and whose sex is Female; LM = large type and whose sex is Male.

CHAPTER FIVE

5.0 DISCUSSION

5.1 Nutrient analysis of Moringa oleifera

The similarities in chemical composition of Moringa leaf meal in this study with those of Kakengi et al. (2007) and Becker, (1995) indicate that environmental factors play a minor role in determining nutritive value of Moringa leaf meal. The Moringa tree can thus be propagated in any part of the country. The chemical compositions of other feed ingredients used in the formulation of experimental diets were within the ranges reported in literature (McDonald et. al., 1995).

5.2 Mean Feed consumption

The findings that there were no significant differences ($P>0.05$) for the mean feed consumption in experiment 1 are in agreement with the observations of Kakengi et al. (2007), that diets with Moringa leaf meal inclusion are preferred by chickens. Weekly feed intakes in experiment 2 were high, but varied according to sex on all diets and differences were significant ($P<0.05$). Results obtained in the two experiments on dry matter intake have therefore revealed that diets having Moringa leaf meal are preferred by indigenous Zambian chickens. Although anti-nutritional factors and toxic materials were not measured in this study, high feed intakes suggest lower anti-nutritional factors and toxic materials in Moringa leaf meal according to the findings of Makker and Backer (1997) and Ravindran et al., (1986).

5.3 Performance of the Birds

In the first experiment, there were significant differences ($P<0.05$) in body weight means between treatments M0 and M10 as shown in Table 4. It was interesting to note that the indigenous chickens on M10 had the highest feed intake as well as digestibility leading to an increased growth pattern. The significant results ($P<0.05$) thus obtained from this study confirm previous findings that indicated that Moringa Leaf Meal promoted good growth and productivity in poultry attributed to its nutrients and phytochemicals (Kakengi et.al 2007). However, Kakengi et al. (2007) experimented on laying hens. Responses to different levels of Moringa Leaf Meal in the diets were observed and these were similar to those reported by Afuang et al., (2003) on the effect of Moringa Leaf Meal on female mice and in Nile Tilapia.

5.4 Regression analysis between amount of *Moringa oleifera* in the feed and intestinal microbial load

When the regression coefficient value obtained during experiment one (0.3282) is multiplied by 100, it gives the percentage of the antibacterial principle that is accountable for decreasing the microbial population. In this case, 32.82% is the fraction of the antibacterial principle that accounts for the decrease in the microbial population. The coefficient of correlation between the amount of Moringa in the feed and the intestinal microbial load indicates a negative relationship with an r value of 0.5. Though being a moderate relationship, it shows that an increase in the percentage of Moringa leaf meal in feed decreases the microbial population. Despite treatment M10 having more colonies of the microbes than M20 and M30, it can be deduced that treatment M10 had the antibiotic principle falling within the recommended amounts

of 0.5-3µg/cc, Fahey (2005). This is so because at this recommended level, the antibiotic is able to inhibit gram positive bacteria, gram negative bacteria and micrococcus. The lower microbial load led to an increase in the activities of digestive enzymes and improved feed efficiency as intestinal bacteria are known to produce growth-depressing metabolites and tend to alter the maintenance energy requirements. Although the antibiotic principle pterygospermin, it can be assumed that the effects of pterygospermin must have played a major role in influencing the results obtained as have been reported by (Rao et al., 1996, Ruckmani et al., 1998, Das et al., 1957 and Caceres et al., 1991). The bacterial standard set for these tests were 10^5 and $10^{6.}$. The maximum recovery from the present study was therefore within the microbiological specifications.

5.5 Apparent digestibility

The apparent digestibility results obtained from the indigenous chickens on M10 are high (81% and 80% for protein and energy respectively). These results agree with those reported by (Martin et al., 1998; Mupeta et al., 2002; Poppi and McLennan, 1995) who reported leaf apparent digestible protein of between 80% and 87% in Moringa leaf meal. This shows that Moringa leaf meal is a good source of energy and protein. The low digestibility results observed from the birds on M20 and M30 can thus be attributed to high crude fiber content in the feed. It was however observed that the feed intake and acceptability of the feed reduced at higher levels (>10%) of Moringa leaf meal inclusion in the diets. This may also explain why only the birds on M10 had significantly high weight gains as the intake was highest for the chickens on M10.

5.6 The effects of Moringa oleifera leaf meal on blood antibody levels of indigenous Zambian chicken type/sex.

In this study, birds on treatment M10 had less mortality in both experiment one and two. It can be assumed that there was better control of the multiplication and spread of parasites present in the body system for birds on M10 and can be attributed to an efficient response system of the various kinds of effector cells, with T-cells being fundamental to this control. These cells secrete cytokinines that act on effector cells to enhance their cytotoxic or cytostatic capabilities and increase cell numbers (Ray-Yu Yang1 et.al 2006; Caceres et.al 1991). In poultry, the two central organs in the avian immune system are the thymus (T-cells) and the bursa of fabricius (B-cells).

There were no differences ($P>0.05$) between treatment means for antibody titre levels before and 7 days after vaccinating against Newcastlle disease. In both scenarios, the M10 treatment had antibody titres at higher but insignificant ($P<0.05$) levels. This could explain why the chickens on this dietary treatment in both experiments were able to resist infections and mortality was lower than those on other treatments. It is well known that one of the functions of white blood cells is to protect the body from infection by enhancing the production of antibodies apart from destroying virus infected cells and engulfing foreign bodies. This clearly indicates that Moringa elicits the production of white blood cells such that their levels tend to be slightly above the normal levels. Hence, this tend to substantiate what has been reported by Jayavardhanan et al, (1994) and Fuglier, (1999) that Moringa boosts the immune systems.

CHAPTER SIX

6.0 CONCLUSION

The two experiments conducted give clear indications that Moringa Leaf Meal promotes good growth in indigenous chickens attributed to its nutrients. This is due to the fact that at 10% inclusion in a diet, Moringa Leaf Meal promotes fast growth attributed to its high acceptability and digestibility. This study has also shown that 10% of Moringa Leaf Meal in the diet confers immunity to the indigenous Zambian chickens by reducing the microbial load in the intestines. The reduced microbial load can be attributed to the antimicrobial activity of Pterygospermin as evidenced by low mortalities of 5% and 12% for experiment one and two respectively for indigenous Zambian chickens receiving 10% inclusion levels of the Moringa Leaf Meal. The higher antibody titres levels for the indigenous chickens on 10% Moringa Leaf Meal diet even before being vaccinated against Newcastle disease further amplifies what has been reported that Moringa Leaf Meal improves the immunity of indigenous chickens through its natural antimicrobial compounds. Finally, the digestibility tests have revealed that apparent digestibility for protein and energy was better in diets having 10% Moringa Leaf Meal and is readily digested and utilized in the chicken's body system.

CHAPTER SEVEN

7.0 RECOMMENDATIONS

Traditional poultry farmers should be encouraged to use Moringa leaf meal in their poultry feed. Results obtained from this study have showed that 10% inclusion rate of Moringa leaf meal in poultry diets gave the best growth and health performance to indigenous Zambian chickens. The Ministry of Livestock and Fisheries development should come to the forefront of promoting the use of non conventional feedstuffs like Moringa to poultry farmers in the country in its quest to contribute to rural wealth creation through enhanced rural poultry production. Moringa is cheap and can be readily available once the Moringa oleifera trees are planted in large numbers.

Traditional poultry farmers must therefore be made aware of Moringa and its rich nutrient and antibacterial compounds. As many livestock households as possible must be encouraged to grow at least one tree. This would ensure a ready supply of feed and antibiotics for the chickens even during the dry season as the tree is drought resistant.

Research should be conducted to investigate further the exact inclusion level between 6% and 14% at which Moringa would give the best performance in terms of growth and health.

REFERENCES

Adene, D.F. (1996). International poultry health problems: Perspective from the poultry industry in Africa. In Proceedings, 20th World Poultry Congress, New Delhi, India, 1–5 September 1996, Vol. 2, p. 401–414.

Afuang, W., P. Siddhuranju and K. Becker, (2003). Comparative nutritional evaluation of raw, methanol extract residues and methanol extract of Moringa (*Moringa oleifera* Lam.) leaves on growth performance and feed utilization in Nile Tilapia. (*Oreochromis niloticus* L.). Aquaculture Res., 34:1147-1159.

AOAC (1990). Association of Agricultural Chemists Official methods of Analysis, 15th Edition. Arlington, Virginia, USA. pp 807- 809.

Becker, K., 1995. Studies on utilization of Moringa oleifera leaves as animal feed. Institute for Animal Production in the Tropics and Subtropics, vol. 480. University of Hohenheim, Stuttgart, p. 15.

Bell, J.G., Kane, M. & Le Jan. C. (1990). An investigation of the disease status of village poultry in Mauritania, Preventive Veterinary Medicine, 8(4): 291–294.

Bhatnagar R, Kataria M and Verna S V S (1996) Effect of dietary Leucaena leaf meal on the performance and egg characteristics in white leghorn hens. Indian Journal of Animal Science 66 (12): 1291-1294.

Bhatnagar SS, Santapau H, Desai J.D.H, Yellore S, Rao TNS. (1961). Biological activity of Indian medicinal plants. Part 1.Antibacterial, antitubercular and antifungal action. Indian J Med Res **49**: 799–805.

Bourzat, D. & Saunders, M. (1990). Improvement of traditional methods of poultry production in Burkina Faso. In Proceedings, CTA Seminal; 3rd International Symposium on Poultry Production in Hot Climates, Hame1n, Germany, 12 June 1987.

Caceres A, Cabrera D. Morales O, Mollinedo P, Mendia P, (1991), Pharmacological properties of *Moringa oleifera* 1: preliminary screening for antimicrobial activity. Towrial of Ethnopharmacology, 33, 213-216.

Chaheuf, N. (1990). Disease prevention in smallholder village poultry production in Africa. In Proceedings, CTA Seminar on Smallholder Rural Poultry Production. Thessaloniki. Greece, 9–13 October 1990, Vol. I, p. 129–137.

Chrysostome, C.A.A.M., Bell, J.G., Demey, F. & Verhulst, A. (1995). Seroprevalences to three diseases in village chickens in Benin. Preventative Veterinary Medicine, 22(4): 257–261

Das BR, PA Kurup, PL Narasimha Rao (1957) Antibiotic principle from *Moringa pterygosperma.* VII. Antibacterial activity and chemical structure of compounds related to pterygospermin. *Indian Journal of Medical Research* 45: 191-196.

Duke JA (1987) Moringaceae: Horseradish-tree, benzolive-tree, drumstick-tree, sohnja, moringa, murunga-kai, malunggay, p. 19-28. In: M. Benge (ed.) Moringa: A multipurpose vegetable and tree that purifies water. Sci. & Technol./ For., Environ., & Natural Resources Agro-Forestation Tech. Ser. 27. US AID, Washington, D.C.

Eilert U, B Wolters, A Nahrstedt (1981) The antibiotic principle of seeds of *Moringa oleifera* and *Moringa stenopetala*. *Planta Medica* 42: 55-61.

Fahey J.W. (2005). *Moringa oleifera*: A review of the medical evidence for its nutritional, therapeutic, and prophylactic properties. Trees for Life Journal 1: 5

Fuglie LJ (1999) The Miracle Tree: *Moringa oleifera*: Natural Nutrition for the Tropics. Church World Service, Dakar. 68 pp.; revised in 2001 and published as The Miracle Tree: The Multiple Attributes of Moringa, 172 pp. http://www.echotech.org/bookstore/advanced_search_result.php?keywords=Miracle+Tree.

Gueye, E.F. (1997). Senegalese poultry industry expands with various bottlenecks. World Poultry 13:10-11

Haazele, Maimbo,Musukwa and D Lungu (2000). Manual for Effective Management of Village chicken to Enhance Production, Smallholder Enterprise and Marketing Programme, Lusaka, Zambia.

Ivan Roitt, Jonathan Brostoff, David Male (Eds.)., (1993). Immunology, 3rd Edition., Mosby Publication.

Jayavardhanan KK, K Suresh, KR Panikkar, and DM Vasudevan (1994) Modulatory potency of drumstick lectin on the host defense system. *Journal of Experimental Clinical Cancer Research* 13(3): 205-209.

Kakengi A M V, Kaijage J T, Sarwatt S V, Mutayoba S K, Shem M N and Fujihara T (2007). Effect of *Moringa oleifera* leaf meal as a substitute for sunflower seed meal on performance of laying hens in Tanzania. *Livestock Research for Rural Development. Volume 19, Article #120.*

Makkar H P S and Becker K (1996) Nutritional value and anti nutritional components of whole and extracted Moringa oleifera leaves. Animal Feed Science and Technology 63, 211 – 228

Makkar, H.P.S. and Becker, K., (1997). Nutrient and anti quality factors on different morphological parts of the Moringa tree. *Journal of Agricultural Science* 128: 311 - 322.

Martin FW, RM Ruberte, and LS Meitzner (1998) Edible Leaves of the Tropics. 3rd Ed. Educational Concerns for Hunger Organization, Inc., N. Ft. Meyers, FL. 194 pp.

McDonald P, Edwards R A, Greenhalgh J F O and Morgan C A (1995) Animal nutrition 4th edition. John Wiley and sons, United States. 607 pp.

Mehta R, Nambiar R.G. Delgado C. L., Subramanyam S. (2003).Policy, Technical and Environmental Determinants and implications of the scaling-up of Broiler and egg production in India Final Report of IFPRI- FAO Livestock Industrisation Project: Phase II. IFPRI, Washingston DC and FAO,Rome.

Mupeta, B., Wood, J., and Mhlanga, J. (2002). The performance of village chickens under improved feed management compared with the hybrid chickens in Zimbabwe. DFID project R7527. NR International, Aylesford, Kent, Uk.

Nakamura, H., Narita, M., Kawamura, K., Shirai, J., Furuta and Abe, F. (1990). An Immuno-histological Study on the nephritis in chicks experimentally produced with avian nephritis. *Avian pathology 19:497-509.*

Nikkon, Saud F.A, Rahman M.H and Haque M.E., (2000). In-vitro antimicrobial activity of the compound isolated from chloroform extract of *Moringa oleifera* Lam. Pakistan Journal of Biological Science, **6** (22): 1888-1890.

Osei S A, Opuku and Atuahene C C (1990). Gliricidium leaf meal as an ingredient in layer diets. Animal Feed Science and Technology 29:303-308.

Permin .A., and Bisgaard, M. (1999). A general review on some important diseases in free range chickens. Proceedings of a workshop, March 22-26, Tune Landboskole, Denmark (in Press).

Poppi, D.P., McLennan, S.R., 1995. Protein and energy utilization by ruminants at pasture. J. Anim. Sci. 73, 278– 290.

Rao K.S., Misra S.H.(1998). Anti-inflamatory and anti-hepatotoxic activities of rats on *Moringa* pterygosperma. *Indian Journal of pharmacological Science. Vol 60: 12 - 16.*

Ravindran V, Kornegay E T, Rajaguru A S B, Potter L M and Cherrry J A (1986). Cassava leaf meal as a replacement for coconut oil meal on broiler diets. Poultry Science 65(9): 1720- 1727.

Ray-Yu Yang1, Lien-Chung Chang, Jenn-Chung Hsu, Brian B. C. Weng, Manuel C. Palada, M. L. Chadha and Virginie Levasseur, (2006). Nutritional and Functional Properties of Moringa Leaves-From Germplasm, to Plant, to Food, to Health. Nutrition, Plant Breeding, and Crop and Ecosystem Management, AVRDC- The World Vegetable Center, Shanhua, Tainan, Taiwan, ROC.

Rocha, M.L.R., Mendieta, B., 1998. Efecto de la suplementacio´n con follaje de Moringa oleifera sobre la produccio´n de leche de vacas en pastoreo. Tesis Ing. Agron. Facultad de Cienci Animal. Universidad Nacional Agraria Nicaragua, Managua.p. 36.

Ruckmani K., Kavimani S., Anandan R., Jaykar B. (1998). Effect of *Moringa oleifera* Lam on paracetamol-induced hepatoxicity. *Indian Journal of Pharmacological Science – 60: 33-35.*

Sarwatt S V, Kapange S S and Kakengi A M V (2002) The effects on intake, digestibility and growth of goats when sunflower seed seed cake is replaced with Moringa oleifera leaves in supplements fed with Chloris gayana hay. Agroforestry systems Volume 56:241-247

SAS Institute Inc. (1998) SAS/ STAT Users Guide release 6.03 Edition. Carry, Inc., 1028 pp

Smith A J (1999). Poultry. The tropical agriculturist First edition. Macmillan Publishers. Wageningen. 218 pp.

Smith, A.J., 1990. Poultry. CTA - Macmillan Publishers Ltd, London, pg. 11, 179-191.

Snedecor, G.W. and W.G. Cochran, (1992). Statistical Methods 7th edition. The Iowa State University Press. Iowa. US.A.

Spradbrow, P.B., Jayawardane, G.W.L. (1993-94). Cell Mediated Immunity in chickens Vaccinated with the V4 strin of New Castle Disease Virus. *Journal of Poultry Science. Volume 72: 7.*

Tangendjaja B, Rahardjo V C and Lowry J. B. (1990). Leucaena leaf meal in the diet of growing rabbits: Evaluation and effect of a low mimosine treatment. Animal Feed Science and Technology 29: 63-72.

Tona, G.O. (1995). Incidence of worms in chickens on farms in Ikorodu Local Government Area of Lagos State, Nigeria. *African Network of Rural Poultry Development Newsletter*, 5(1).

Vohra P (1972). Evaluation of metabolizable energy for poultry. World's poultry Science Journal 28: 204-214.

Yongolo, M.G.S. (1996). *Epidemiology of Newcastle disease in village chickens in Tanzania.* PhD dissertation, Sokoine University of Agriculture, Morogoro, United Republic of Tanzania.

Zaria, T., Sinha, P.K., Natiti, L.S. & Nawathe, D.R. (1993). Ectoparasites of domestic fowl in an arid zone. *African Network of Rural Poultry Development Newsletter*, 3(2): 7.

APPENDICES

APPENDIX A*:*

Appendix A1: Effects of Moringa oleifera leaf meal on body weights of indigenous Zambian chickens in grams

WEEK	M0	M10	M20	M30
0	314.67	312.00	315.33	291.33
1	374.00	365.33	346.66	356.42
2	398.57	387.33	337.85	402.30
3	426.15	459.33	371.42	440.76
4	444.30	515.20	381.28	440.30
5	473.77	606.73	425.81	493.83
6	562.33	606.73	425.81	493.83
7	568.71	621.20	460.00	520.33
8	592.00	596.66	445.45	533.45
9	664.33	613.53	508.80	540.81
10	714.83	699.76	525.80	601.10
11	820.16	750.07	575.77	626.90
12	890.66	972.43	627.11	640.03

Appendix A2: Effects of Moringa oleifera leaf meal on mean feed consumption of indigenous Zambian chickens (in grams)

WEEK	M0	M10	M20	M30
1	450.00	420.00	426.66	325.00
2	259.33	193.33	167.16	212.22
3	318.66	334.00	316.00	215.33
4	300.16	251.46	249.26	263.55
5	280.63	224.80	128.80	190.03
6	453.91	282.33	209.86	361.21
7	385.72	189.73	250.73	217.32
8	210.88	249.13	360.15	245.80
9	406.33	241.00	263.70	240.97
10	458.00	470.42	451.54	415.75
11	474.00	354.23	300.40	299.76
12	398.50	391.31	345.36	173.82

APPENDIX B:

Appendix B1: Blood antibody levels in micro liters before vaccinating against Newcastle disease

FEED	LARGE MALE (LM)	LARGE FEMALE (LF)	DWARF MALE (DM)	DWARF FEMALE (DF)
M5	116	114	127	163
M10	333	228	342	214
M15	224	269	168	160

Appendix B2: Blood antibody levels in micro liters 7 days after vaccinating against Newcastle disease

FEED	LARGE MALE (LM)	LARGE FEMALE (LF)	DWARF MALE (DM)	DWARF FEMALE (DF)
M5	533	552	580	588
M10	1203	601	851	662
M15	530	566	517	503

Appendix B3: Blood antibody levels in micro liters 14 days after vaccinating against Newcastle disease

FEED	LARGE MALE (LM)	LARGE FEMALE (LF)	DWARF MALE (DM)	DWARF FEMALE (DF)
M5	749	867	736	781
M10	1021	1039	1005	1051
M15	976	843	808	880