The Effect of *Moringa oleifera* Leaf Meal Supplementation on Growth and Health of Village Chickens

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ABSTRACT

Objectives and Study Design: In a quest to intervene in high mortality incidences and poor growth among village chickens in Zambia, two experiments were carried out to study **the effect of *Moringa oleifera* leaf meal supplementation on growth and health of village chickens.** In experiment one, a total of sixty village chickens were blocked by body weight and 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. 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. In experiment 2, a total of 60 village chickens were divided equally between sex and type into 12 groups and assigned at random to three isonitrogenous and isocaloric dietary treatments containing 5%, 10% and 15% Moringa 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. Blood samples were taken from the wing veins using sterile needles for antibody titre level analysis using the Enzyme Linked Immuno Sorbent Assay method.

Results: Results from experiment one revealed that growth were 41% and 49% ($P<0.05$) higher in Village chickens receiving diets with 0% and 10% Moringa leaf meal, respectively than those receiving 20% and 30%. There were significant differences ($P<0.05$) in the faecal bacteria count with diets containing 10% Moringa leaf meal having the lowest counts. The results from experiment two revealed that 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 1000µL whereas those on M5 and M15 had 783 and 876, respectively. For the chickens on M10, this 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. There were no differences ($P>0.05$) between treatment means for antibody titre levels before and 7 days after vaccinating against Newcastle disease. In both scenarios, the M10 treatment however, had antibody titres that were at higher and insignificant ($P<0.05$) levels. Differences ($P<0.05$) occurred 14 days after the vaccination, with M10 having the highest antibody titre levels. This explains why the chickens in both experiments were able to resist infections and had less mortality than those on other treatments.

Conclusion: The results from the two experiments indicate that 10% inclusion of Moringa leaf meal in the diet promotes growth and optimum utilization of the natural antimicrobials/herbal medicines.

INTRODUCTION

In Zambia, more than 93% of the population in the rural areas own village chickens (9). These village chickens can be sold or bartered to meet essential family needs such as medicines, clothes and school fees (8, 23). The major constraint of village 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 (18).

Recent studies have demonstrated that Moringa leaf meal provides natural antimicrobials/herbal medicines, vitamins, minerals, and proteins (22). Moringa plant is drought resistant and grows in all types of soils ranging from acidic to alkaline (6), and at altitudes from 0 to 1800 m above sea level. 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-15tons/ha/year. In a study in Nicaragua, the fresh leaves were found to contain 23% crude protein (CP) in DM, 12.3 MJ/kg DM and had an *in vitro* DM digestibility of 79.7% (3). Moringa is also rich in carotenes, ascorbic acid, iron and in the two amino acids, generally deficient in other plant feeds, i.e. methionine and cystine (12) and are required at 0.28% and 0.31% in poultry diets, respectively. Reduced blood triglycerides and enhanced immune response has been reported (21) due to increased peripheral and splenocyte T-cell proliferations in rats as a result of Moringa leaf meal inclusion in the diet. Fast growth in man and livestock has been attributed to Moringa being able to promote metabolism with its bio-available ingredients and their ability to increase the natural defenses of the body (5).

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Key words: *Moringa leaf meal, village chickens, digestibility, microbial load, blood antibody titre levels*

Problem statement

Although knowledge has been acquired with regard to the nutrient contents and natural antimicrobials/medicinal properties of Moringa *through numerous studies, its potential for use as a supplement in the diets of village chickens is yet to be explored.*

The main objective of this research was to assess the effectiveness of Moringa as a feed supplement on the productivity of village chickens so as to contribute to rural wealth creation through enhanced rural poultry production by application of Moringa as unconventional feed supplement.

MATERIALS AND METHODS

Source of Moringa Leaf Meal

Moringa leaves were harvested from an orchard within Lusaka District (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.

Experiment One

Experiment one was conducted at Mazabuka Livestock Research Institute (National Artificial Insemination Services) in Southern Province. A total of sixty village chickens, of mixed type each weighing 110 grams on average were purchased from a local farmer in Kasisi, Lusaka. The village chickens had been hatched from a homemade incubator and were two weeks of age at the time they were bought. After an adaptation period of two weeks, the immature chickens were equally divided into three groups of 20 according to body weight to form blocks. Group one had chickens weighing between 105 – 108 grams, group two 109 – 111 grams and group three had chickens weighing between 112 – 114 grams. The three groups were further divided into four groups of 5 chickens and were assigned at random to four dietary treatments. At this time, the chickens were four weeks of age and weighed 308 grams on average. The treatments had 0%, 10%, 20% or 30% of Moringa leaf meal on weight basis. The diets were isonitrogenous and isocaloric. The experimental design was a 4x3 Randomized Complete Block Design with four diets and three replicates aimed at removing weight variations among and within the groups. In this experiment, the chickens were the experimental unit, the four formulated diets were the treatments, and the blocking factor was the body weights. Four isonitrogenous and isocaloric experimental village chicken diets were formulated to obtain diets having 0%, 10%, 20% and 30% of Moringa leaf meal on weight basis designated as M0, M10, M20 and M30, respectively. The experimental period was twelve weeks. Prior to the commencement of the trials, a week was used to allow the chickens to adapt to the diets.

Biochemical analysis

Samples of Soyabean meal and number 3 maize meal sourced from Livestock Services in Lusaka and Moringa leaves were subjected to proximate analysis according to the Association of Official Analytical Chemists (1) methods. All the analysis were done in triplicate.

Data collection

The body weights were taken as absolute weights of the individual birds at 06:00 hrs in the morning and this was done with the use of a sensitive balance (0.005g) having a capacity of three decimal places. Feed intake was determined by subtracting the final weight of the feed in the trough from the initial weight.

Bird mortality was recorded and the carcasses were taken for postmortem examination at the veterinary clinic in Mazabuka District.

For apparent digestibility tests, fresh feacal samples were collected from each pen and put in plastic capsules. The capsules were labeled accordingly and put in a cooler box. The samples were then taken to the Animal Science Laboratory at the University of Zambia. In the laboratory, the samples 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).

Statistical analysis

The data generated and laboratory results collected for both experiments were subjected to Analysis of variance using general linear model (GLM) procedures of Statistical Analysis System (SAS) Inc, (23). T-test analysis was done for final mean live-weights. Regression analysis was done between the percentage of Moringa leaf meal and intestinal microbial load. Values were considered significant at $P<0.05$. Treatment means were separated by using Duncan's multiple range test - least square means (Lsd), (23).

Experiment Two

Experiment two was conducted at the University of Zambia (Great East Road campus) School of Agricultural Sciences Field Station livestock unit. The aim was to evaluate further the effects of Moringa leaf meal on the health of village chickens at dietary inclusion levels of less than 20%. This was arrived at based on indications from experiment one that chickens receiving 10% of Moringa leaf meal had the least mortality rates. 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). Only the 10% diet was maintained while 5% and 15% were used in order to explore further if there were better results other than at 10% whose performance was known with respect to experiment one. The diet having 10% of Moringa leaf meal gave the best growth and health performance during experiment one and was used as the reference diet. In this

experiment, sixty village chickens were divided equally between sex and body size. The chickens were sourced from the same farmer as in experiment one. Unlike in experiment one, 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. The Moringa leaves were harvested from an orchard within Lusaka (Makeni area). Its preparation procedure is as explained under experiment one.

Management of the chickens

The chickens in this trial were kept in cages of 5 birds per pen. They had *ad libitum* access to feed and fresh water. Fresh water was supplied every day. Required managerial practices were also applied as and when due.

Blood collection and analysis

Blood samples were obtained from three birds 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 millilitres 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.

MAIN STUDY FINDINGS

Experiment One

Nutrient analysis of Moringa leaf meal, soyabean meal and number 3 maize meal.

The results for the nutrient analysis done on Moringa leaf meal, soyabeans and number 3 maize meal at the University of Zambia's Animal Science Laboratory are presented in Table 1. The ingredients had an average dry matter (DM) content of 89.39±1.

Table 1: ***Proximate composition of Moringa oleifera leaves, Soyabean meal and Maize meal used in experiment one***

Nutrients/Parameters	Moringa	Soyabean Meal	Mai ze Meal
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

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

Effect of Moringa oleifera leaf meal on growth performance of village chickens

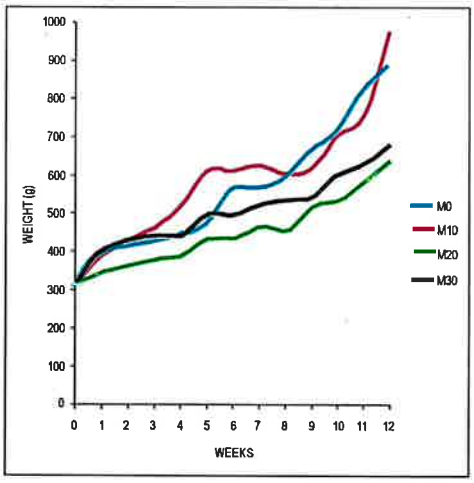
Trends in the growth of the chickens are illustrated in Figure 1. At the end of the experiment, there were significant differences ($P<0.05$) between treatments M0, M10 and M30 with regard to final body weights. Treatment M10 had a value of 972.43 grams and was significantly different from M20 and M30 that had values of 646.78 grams and 640.03 grams, respectively. 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 more 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. Variations in growth patterns for chickens on M30 were observed in the fourth week and were lower than those for the chickens on M0 and M10. The chickens on M20 had the lowest growth pattern with more growth variations until after week ten. Table 4 shows the T-Test analysis results for final mean live-weights.

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

Figure 1: *Weekly live-weights for village chickens receiving the four experimental diets*



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

Effect of Moringa oleifera leaf meal on feed intake of village chickens

The results obtained revealed that there were no significant differences in the total DM intake of village chickens on the different treatments ($P<0.05$) but the trend was that village chickens on M0 had the highest DM intake (398.5 g/day) followed by M10 (391.3 g/day) and M20 (345.4 g/day), while M30 village chickens had the lowest intake (173.8 g/day).

Effect of Moringa leaf meal on intestinal microbial load of village chickens

Figure 2 shows the results for regression analysis **between the percentage of** Moringa **leaf** meal and intestinal microbial load with a regression coefficient value of 32.82%. The coefficient of correlation between the amount of Moringa in the feed and the intestinal microbial load revealed a negative relationship with an r value of 0.5. Table 2 shows the effects of oleifera leaf meal on microbial colonies per gram of feacal sample.

Figure 2: ***Regression analysis between the percentage of Moringa oleifera leaf meal and intestinal microbial load***

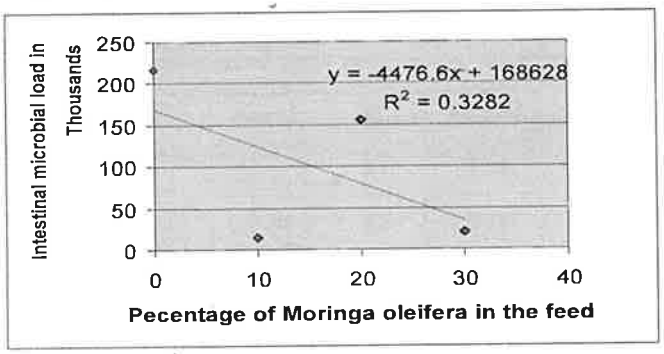


Table 2: ***Effects of Moringa oleifera leaf meal on microbial colonies per gram of feacal sample***

	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

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

Effect of Moringa 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. Crude protein apparent digestibilities for M0 and M10 (80% and 81% respectively) were significantly ($P<0.05$) higher than those for M20 and M30 (68% and 75% respectively). The apparent digestibilities for phosphorus for treatments M10 and M30 were 81% and 74%, respectively and were significantly ($P<0.05$) higher than those for M0 and M20 (66% and 49%, respectively). The apparent digestibilities for M0, M10 and M20 for ether extracts were numerically higher than those for M30 while the digestibilities for calcium for M0, M10 and M30 were also numerically higher than those for M20. However, the results for ether extract and calcium did not show any statistical differences ($P<0.05$).

Experiment Two

Nutrient analysis of Moringa leaf meal, soyabean meal and number 3 maize meal

The results for the nutrient analysis done on Moringa leaf meal, soyabeans and number 3 maize meal at the University of Zambia's Animal Science Laboratory were: Metabolisable energy was highest in Maize (3470 Kcal/Kg) followed by Moringa (3271 Kcal/Kg) and Soyabeans (2982 Kcal/Kg). Crude Protein content was 34%, 26.61%, and 8.5% for Soyabeans, Moringa and Maize, respectively. The results for the other parameters measured are presented in Table 3.

Table 3: ***Proximate Composition of Moringa oleifera leaves, Soya beans and Maize meal used in experiment two***

	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

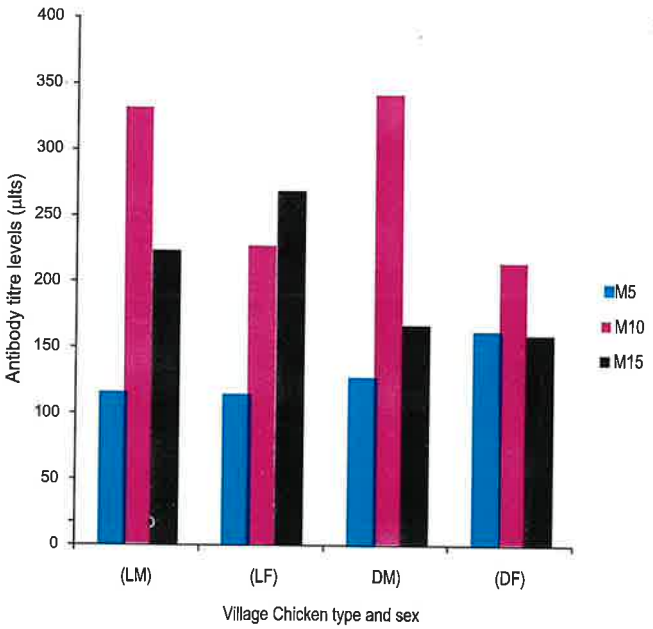
MOLM = *Moringa oleifera* leaf meal, DM = Dry Matter, ME = Metabolisable Energy, CP = Crude Protein, CF = Crude Fibre, EE = Ether Extract, Ca = Calcium, P = Phosphorus.

Effect of Moringa leaf meal on blood antibody levels of village chicken type/sex

There was no differences ($P<0.05$) between treatment means for antibody titre levels before and 7 days after inoculating the chickens with Newcastle virus as in Figures' 3 and 4. Antibody titre levels 14 days inoculating the chickens with Newcastle virus are presented in Figure 5. The results from M10 differed significantly ($P<0.05$) from M5 and M15. The mean value for

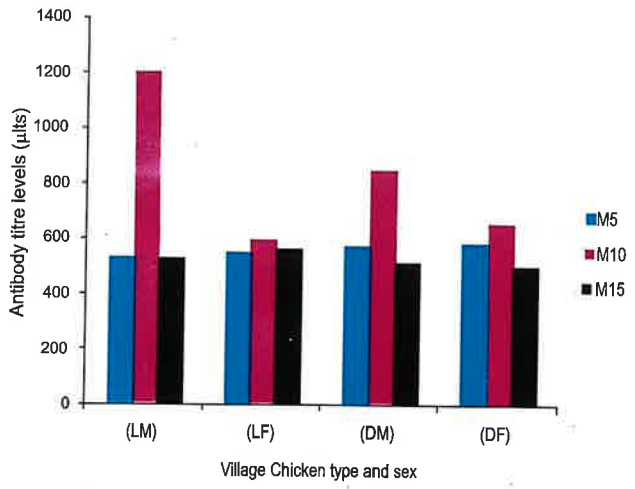
M10 was 1029 micro litres which was higher than the values of 783 and 876 micro litres for treatments M5 and M15, respectively.

Figure 3: ***Results for the first blood antibody titre levels tests conducted on the village chickens***



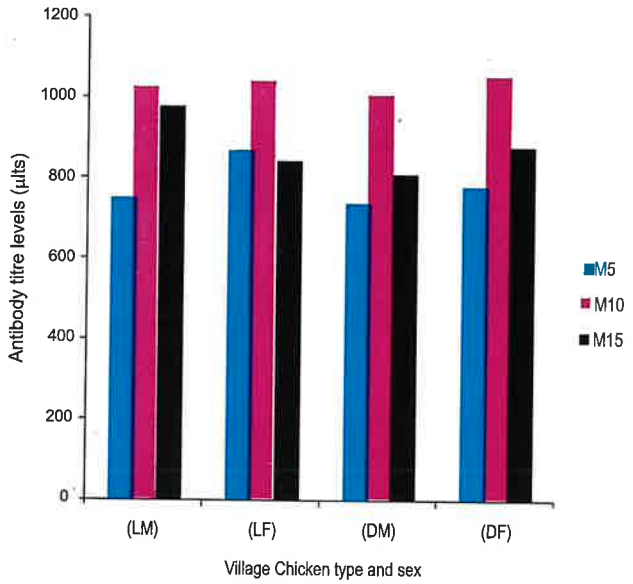
Note: DF = Dwarf type and whose sex is Female; DM = Dwarf type and whose sex is Male; LF = large type and whose sex is Female; LM = large type and whose sex is Male

Figure 4: ***Results for the second blood antibody titre levels tests conducted on the village chickens***



Note: 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 5: ***Results for the third blood antibody titre levels tests conducted on the village chickens***



Note: 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.

Chicken Mortalities

A total of seventeen out of sixty chickens died during experiment two. Chicken mortalities in percentages were 53%, 12% and 35% for M5, M10 and M15, respectively. This was after the chickens were challenged with the Newcastle virus.

DISCUSSION

Nutrient analysis of Moringa leaf meal

The crude protein (CP) content of the Moringa leaf meal used (26.61% as in Table 1) was higher than the CP value of 23.27 % Moringa leaf meal reported by Makkar et al. (12). The values obtained for the ether extract (EE), calcium (Ca) and Phosphorus (P) for the Moringa leaf meal in the present study were similar to those reported by Oduro et al. (19). The crude fibre content of 3.60% observed however, contradicts Yang et al. (21) who reported crude fibre content of 19%. According to Fuglie (7), variations in protein and crude fibre contents can be attributed to the age of cutting or harvesting, climatic conditions, edaphic factors, agronomic practices as well as methods of processing and analysis. The CP of Moringa leaf meal as observed was however, lower than that of soyabean meal (44%) and fish meal (60%) used conventionally as sources of protein in poultry rations.

The chemical compositions of other feed ingredients used in the formulation of experimental diets as indicated in Tables 1 and 3 were within the recommended levels as reported by McDonald et al. (14).

Growth performance of the birds

The higher weight gain of village chickens fed with the diet containing 10% Moringa leaf meal suggests that this level may have stimulated higher digestion of the nutrients consumed resulting in greater efficiency in the utilization of feed resulting in enhanced growth. Fuglie (7), Jayavardhanan et al. (10) and Kakengi et al. (11) had earlier reported that Moringa leaf meal possess digestion stimulating properties attributed to its nutrients and phytochemicals. The weight gain for M20 and M30 were low and this can be related to the findings of Bouatene et al. (4) who reported that Moringa leaf meal contains an excess of methionine and hence leads to low appetite. Further, the researcher reported that excess methionine would have a net depressive effect on consumption and weight gains.

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

The coefficient of correlation of 32.82% (Figure 2), obtained in this study indicates a negative relationship with an r value of 0.5, showing that an increase in the percentage of Moringa leaf meal in feed decreases the microbial population. The comparable high number of microbial colonies ($2.165X\ 10^7$) for treatment M0 with respect to M10, M20 and M30 can be deduced that treatment M10 had the antibiotic principle falling within the recommended amounts of 0.5-3µg/cc. This observation is in agreement with Moyo et al. (16) and Yang et al. (21) who have reported that Moringa leaf meal exhibited antimicrobial properties through the inhibition of the growth of both gram positive and gram negative bacteria isolated from food and poultry intestines.

Apparent digestibility

The comparable high digestibility results obtained for protein and energy (81% and 85.15% respectively) for M10 and (80% and 89.56% respectively) for M0 indicates that Moringa significantly enhances duodenum traits by having an increased number of ileum microflora that lead to an increased absorption surface area as reported by earlier literature Yang et al. (21). Martin et al. (13), Moyo et al. (16), Mupeta et al. (17) and Poppi et al. (20) have all reported that Moringa leaf meal is a good source of nutrients with leaf apparent digestible protein of between 80% and 87% . However, the low digestibility results observed from the chickens on M20 and M30 can be attributed to the numerically higher crude fibre content of 3.8% and 4.4% respectively in the feed. This could be the reason why the chickens on M0 and M10 had significantly high weight gains as feed intake was high and the crude fibre contents was low (3.55% and 3.4% respectively).

The effects of Moringa leaf meal on blood antibody levels of village chicken type/sex

Blood parameters are important in assessing the quality and suitability of feed ingredients for farm animals and are the major indices of physiological, pathological and nutritional status of an organism Maxwell et al. (15), and Babatunde et al. (2). Changes in the constituent compounds of blood when compared to normal values could be used to interpret the metabolic stage of an animal as well as the quality of feed Babatunde et al. (2). The better control of the multiplication and spread of parasites present in the body system for birds on M10 can be attributed to an efficient response system of the various kinds of effector cells, with T-cells being fundamental to this control. This is consistent with Caceres et al. (5) and Poppi et al. (20) who have reported that these cells secrete cytokinines that act on effector cells to enhance their cytotoxic or cytostatic capabilities and increase cell numbers. In poultry, the two central organs in the avian immune system are the thymus (T-cells) and the bursa of fabricius (B-cells). Sex effect on Moringa leaf meal induced higher antibody production in males than in females at 10%.This explains why the village chickens on treatment M10 in both experiments were able to resist infections and had less mortality than those on other treatments. According to Fuglie (7) and Gueye (8), 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.

Chicken Mortality

The numerically high value observed in the antibody titres of over 1000µL 14 days after inoculating with Newcastle virus for chickens fed M10, inferred that phytochemicals from Moringa were better utilized and assimilated into the blood stream for use by the chickens. It suggests that the immune system of the birds was adequate as observed by the less mortality recorded for chickens on the M10 treatment. This observation is in agreement with the reports of Yang et al. (21) that chickens fed Moringa leaf meal had enhanced duodenum traits, increased concentrations of total globulin, γ-globulin and IgA, lymphocyte ratio, antibody titer to sheep erythrocytes, and delayed type hypersensitivity as well as reduced E. coli and increased Lactobacillus counts in the ileum thus strengthening the chickens immune functions.

CONCLUSIONS

In conclusion, considering the effects of different levels of Moringa leaf meal on weight gain, feed intake, nutrient digestibility, intestinal microbial load and antibody levels of the village chickens, it has been demonstrated that 10% inclusion of Moringa in the diet gave the best results and should be used for village chickens.

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