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APPARENT HARM CAUSED BY DIETARY HIGH LEVELS OF VIRGIN *MORINGA OLEIFERA* SEEDS IN NUTRITION OF POULTRY

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ABSTRACT

An evaluation of Full-Fat Moringa oleifera Seed Meal (FMOSM) was made by feeding including it in the diets to day- old broiler chicks at varying levels, 7.5, 10.0, 12.5 and 15.0%. Investigation was made on the response criteria of performance, serum chemistry and some haematological indices using a completely randomized design. The experimental period was 4-weeks. Dietary FMOSM depressed performance parameters at inclusion levels above 10% relative to the reference diet ($p < 0.05$). The reverse of result on performance was observed on the biochemical indices of blood glucose, serum total protein and albumin levels which increased in values as the inclusion levels of the seed meal increased in diets up to 15% compared with the control diet ($p < 0.05$). Inclusion of FMOSM in diets at higher levels however, elevated the activities of the transaminases, lactate dehydrogenase and creatinine kinase ($p < 0.05$). The blood composition was significantly affected by dietary FMOSM. PCV, WBC, RBC, Hb, platelet, as well as, MCV, MCH and MCHC were low relative to the control diet fed birds. The concentration of the metabolites, total and direct bilirubins in blood serum were significantly increased ($p < 0.05$) in birds fed FMOSM-based diets. In summary, inclusion of FMOSM in its virgin state above 10% elicited deleterious effects on performance, haematology and caused aberrations in enzyme activities.

Keywords: *Moringa oleifera*, broiler chicks, performance, serum chemistry, blood composition

INTRODUCTION

The inclusion of novel proteins from seeds sources in diets for livestock is receiving advanced research works due to the quest in developing and underdeveloped countries of the world to look for alternatives to the limited conventional protein and energy sources to maintain the increasing human population. Besides, non-conventional feedstuffs are relatively cheap and abundant. Incorporation of unconventional protein feedstuffs in diets is aimed at reducing cost and mitigating

the fierce competition among humans, farm animals and industries for the scarce orthodox feedstuffs. Conventional and unconventional protein bearing seeds not only supply the nutrient protein but also other vital nutrients, carbohydrate, fat, fiber, minerals and vitamins including their precursors. The study to investigate the dietary effects of graded levels of full-fat unprocessed *Moringa oleifera* seeds in nutrition of broilers is sequel to finding an alternative protein source to feed livestock.

Moringa is a perennial tree long known for its nutritional, medicinal and industrial uses. It is native to India but cultivated widely in other tropical countries including the Pacific Islands. The tree's numerous nutritional attributes are particularly beneficial to countries facing undernourishment and food scarcity. All parts of the tree are edible providing the essential nutrients in superior nature relative to the other oil bearing seeds. In traditional medicine practices, the leaves and root barks are very useful in curing diseases (Fahey, 2005) Nutrition wise, the tree has more than 100s of uses: the immature pods are nutritious and rich in minerals and vitamins. The leaves, which are non-toxic, can be consumed fresh, cooked or stored for future use (Donovan, 2007) and the seeds containing 30-40% valuable oil is used for human consumption as cooking oil and in industries. Other industrial uses of moringa products include cosmetics, lubrication, illumination, cellulose pulp for cellophane and textiles. Despite these valuable characteristics, moringa tree is found to contain phytochemicals which when consumed in certain amounts either as sole feed or in diet mixtures elicits deleterious effects. Some of the adverse effects of the anti-nutrients/toxins on food animals include the following: Cyanogenic glycosides-when consumed in high quantity produce excess cyanide ions in the body system of the animal known to inhibit several enzyme systems, depress growth through interference with certain essential amino acids and utilization of associated nutrients. CN ions are reported to cause acute toxicity, neuropathy and death (Osuntokun, 1972; Fernando, 1987). CN ions also inhibit cyatochrome oxidase, stop ATP formation and the tissues suffer energy deprivation and death follows rapidly (Kumar, 1992). The strong affinity of cyanide for iron makes it react with the iron in the haemoglobin making it unable to release oxygen to the tissues hence heart beat and respiratory rates increase resulting to death. Tannins cause decreased feed intake in animals, bind dietary proteins and digestive enzymes to form complexes that are not readily digestible (Aletor, 1993). They also cause decreased palatability due to astringency and reduced growth rate caused by their effects on carbohydrates and proteins metabolism (Roeder, 1995). Toxic saponins are harmful in non-ruminants like poultry. At certain concentration in the body, saponins retard growth due to reduction in feed consumption caused by bitter taste. (Cheeke and Shull, 1985; Shqueir et al. 1989). Haemagglutinins (lectins) are present in *M. oleifera* and cause agglutination of red blood cell. They depress animal growth by interfering with the digestion and absorption of nutrients in the gastrointestinal tract (Aletor and Fetuga, 1987). Moringa alkaloids include moringin, moringinine, spirachin and others. Alkaloids cause GIT and neurological disorders (Aletor, 1993). One of their neurological disorders is paralysis. Both moringine and spirachin can prove fatal at high dose of ingestion. Oestrogenic rich principles have been shown to exhibit poor reproductive performance (Aletor, 1993). Uncontrolled use of plant oestrogens

could produce various types of tumours and internal bleeding in animals and man (Price and Fenwick, 1985). Glucosinolates in seeds/kernels of moringa elicit mortality, impair fertility and are toxic to monogastrics even at a low dose. Thermal degradation of moringa seed glucosinolates produces a toxic variety, 4(alpha-L-rhamnosyloxy) phenylacetonitrile which is mutagenic (Villasenor et al. 1989). Phytates and oxalates contained by *Moringa oleifera* are anti-metals which bind minerals like calcium, magnesium, iron, zinc and interfere with their metabolism. Besides the allelochemicals, *Moringa oleifera* is rich in minerals including heavy metals which are either beneficial or toxic at high concentration. This experiment therefore evaluated the direct incorporation of the full-fat seed meal at graded levels in diets for broilers using performance, serum chemistry and blood indices as response criteria.

MATERIALS AND METHODS

20kg dried seeds obtained from a plantation in a northern state of Nigeria, Kano, were processed into meal by pounding using mortar and pestle. The meal after pounding was further sieved to obtain the desired particle size for broiler chicks. 15kg of the seed meal was used to formulate diets for the experiment. Five diets having on average equal energy and protein contents were formulated (NRC1994) made of a maize-soybeans reference diet and diets 2, 3, 4 and 5 containing 7.50, 10.00, 12.50 and 15.00% full-fat *Moringa oleifera* seed meal (FMOSM) respectively. One hundred and twenty (120) day old broiler chicks from a commercial source were used for the experiment and housed in an electrically heated battery brooder cage partitioned in units to enable replication. The chicks were randomly distributed to the five dietary treatments in a completely randomized design. Birds were fed the experimental diets twice daily, 8.00am and 2.00pm. Both feed and water were given *ad libitum* during a feeding trial that lasted for four weeks. The composition of the experimental diets on as fed basis is shown on Table 1.

Table 1a: Percentage composition of the experimental diets on as fed basis (kg/100kg)

Diets Ingredients	1	2	3	4	5
Maize	59.80	57.80	55.80	54.30	51.80
Soybean meal	36.30	30.80	30.30	29.30	29.30
FMOSM	0.00	7.50	10.00	12.50	15.00
Bone meal	2.50	2.50	2.50	2.50	2.50
Oyster shell	0.50	0.50	0.50	0.50	0.50
Salt	0.20	0.20	0.20	0.20	0.20
DL-methionin	0.20	0.20	0.20	0.20	0.20
Premix	0.50	0.50	0.50	0.50	0.50
Total	100.00	100.00	100.00	100.00	100.00

Table 1b: Nutrients (proximate) composition of the experimental diets (%)

Diets Nutrients	1	2	3	4	5
Dry matter	88.10	83.90	89.14	89.56	84.00
Protein	21.00	20.00	20.89	20.34	22.47
Ether extract	4.40	13.15	14.08	14.15	15.02
Fibre	3.61	5.30	5.88	5.90	6.10
Total ash	6.72	6.06	6.71	7.14	8.41
NFE	32.00	38.04	41.43	41.45	43.93
GE (kcal/g)	4.51	4.52	4.46	4.10	4.20

In the course of the experiment, data was collected on performance indices, feed intake, body weight gain, feed efficiency and survival rate. At the end of the trial, one broiler per replicate from all the treatments was randomly taken and sacrificed for blood collection for the determination of serum chemistry and haematological parameters. Blood for haematological analysis was collected in sample bottles containing the anti-coagulant, EDTA while serum chemistry sample bottles were without the anti-coagulant.

Chemical analysis

Total protein was determined by the biuret method using the LABQUEST semi-automatic spectrophotometer while the protein fraction, albumin was determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) using the bromocresol green method. Blood glucose was determined as described by Lamb (1980). The activities of the enzymes, aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinie kinase and lactate dehydrogenase were determined by the methods of Reitman –Frankel, IFCC and pyruvate-lactate methods respectively. Packed cell volumr (PCV), red blood cell (RBC), white blood cell (WBC) and haemoglobin (Hb) were determined using Wintrobe's microhaematocrit improved Neubauer haemocytometer and cyanomethaemoglobin respectively (Schalm and Caroll, 1975). Total bilirubin and direct (conjugated) bilirubin were determined by Jendrassik Grof method (Spencer and Price, 1977). The mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) were calculated as described by Jones and Hunt (1983).

Statistical analysis

All data collected were subjected to a one way analysis of variance and the significant difference between treatment means was estimated by using Duncan's multiple range test as outlined by Steel and Torrie, (1980).

RESULTS

The dietary performance of broilers fed graded levels of FMOSM is shown on Table 2. Feed intake, body weight gain and efficiency of feed utilization were depressed on diets containing FMOSM relative to the control diet ($p < 0.05$). Survival rate however was not affected by the direct inclusion of the FMOSM at graded levels. The effect of feeding virgin FMOSM at different levels in diets on some biochemical determinants is presented on Table 3. All the measured metabolites, glucose, albumin, total plasma protein were significantly ($p < 0.05$) influenced by the dietary FMOSM. The measured indices were highest in birds receiving diets with FMOSM. There was a significant ($p < 0.05$) increase in the value of urea, uric acid and creatinine in the sera of group of broilers offered higher levels of FMOSM. That is 12.50 and 15.00%. Enzyme activities of AST, ALT, alkaline phosphatase, lactate dehydrogenase and creatinine kinase increased in level in the broilers on the moringa based diets with highest activities recorded on the diet containing the highest level of the seed meal, 15%. The haematological parameters examined and their metabolites are presented on Table 4. Moringa based diets caused a significant decrease in the PCV, WBC, RBC, Hb and platelet values ($p < 0.05$). Other haematological indices (MCH, MCHC and numerically the value of MCV followed the same trend being lowest on Moringa diets ($p < 0.05$). There was a significant ($p < 0.05$) increase in the values of total and direct bilirubins on diets containing moringa seed meal while the 15% FMOSM inclusion recorded the highest values of these parameters.

Table 2: Performance of Broilers fed Dietary Virgin MOSM

Diets Indices	1	2	3	4	5
Feed intake (g/b/d)	36.62^{b}	12.69^{a}	10.50^{a}	10.61^{a}	10.61^{a}
Wt gain (g/b/d)	16.76^{b}	6.47^{a}	4.50^{a}	5.0^{a}	3.60^{a}
F/G	2.19^{a}	2.03^{a}	2.40^{a}	2.35^{a}	3.01^{b}
Survival rate (%)	100.00	100.00	100.00	100.00	100.00

a-b mean values in rows not sharing common letters differed significantly ($p < 0.05$).

Table 3a: Effects of Graded Levels of FMOSM on some Biochemical Parameters in Broilers

Diets Parameters	1	2	3	4	5
Glucose (mmol/l)	6.00^{a}	6.50^{a}	6.47^{a}	6.77^{a}	7.93^{b}
Total protein (g/l)	16.00^{a}	21.oo^{b}	26.67^{c}	31.00^{d}	34.50^{d}
Albumin (g/l)	9.00^{a}	13.00^{b}	17.00^{c}	20.00^{d}	21.33^{d}

a-b-c-d mean values in rows not sharing common superscripts are significantly different ($p < 0.05$).

suggesting that inclusion of the seed meal in poultry diets up to 15% is not lethal to the birds.

Blood metabolites examined with regards to glucose, total serum protein and albumin levels were significantly influenced by including FMOSM in diets at graded levels. These parameters were highest in broilers receiving the highest inclusion of the seed meal in diet 5 that is 7.5mmol/l glucose and 34.50 and 21.33g/l serum protein and albumin respectively. The highest values of the indices recorded on the diet 5 suggests good quality protein of *Moringa oleifera* seed since the higher the values the better the quality of the test feedstuff (Eggum, 1970). Serum enzymes are used in evaluation of the liver and muscles functions. They are found in the body in small quantities and are needed for all the metabolic processes or for all the chemical activities within cells to take place. High quantity as recorded on diets with 10, 12.5 and 15% moringa seed meal inclusions (diets 3, 4 and 5) may signify a disease of the liver (hepatitis) or malfunctioning of the organ (Mitruka and Rawnsley, 1977; Rosenthal and Janzen, 1979).

Blood composition data showed that incorporation of dietary FMOSM at 12.5 and 15% levels caused significant decrease in the PCV, WBC, RBC, Hb, platelet as well as MCV, MCH and MCHC in the experimental animals while increasing the concentration of the metabolites, total and direct bilirubins. The toxic effect of moringa phytochemicals might be responsible for the decrease in PVC, WBC, RBC, Hb, platelet and the other blood parameters and for the increase in the total and conjugated bilirubins. Decrease in the haematological parameters indicated that the birds might suffer some type of anaemia. Decline in WBC count values also showed that the fowls could be predisposed to infection relative to their counterpart on the orthodox diet. RBC values on moringa containing diets were low comparable to the reference diet. Since erythrocytes are responsible for the exchange and transportation of gases, oxygen and carbon dioxide in the body and also for the manufacture of haemoglobin, low values suggests reduction in these activities which may predispose the animals to bad health. Adverse effects on performance and blood composition as a result of high rates of FMOSM in diets conformed to the observations of Aregheore (2002) who reported that levels of moringa in diets of livestock above 10% is attended by poor result due to excessive protein levels and the potentially anti-nutritional compounds in moringa such as lectin, alkaloids, glucosinolates, oxalate, saponin, nitrate, phytate, isothiocyanate and others. The findings on haematology following intake of dietary graded levels of FMOSM in its natural state confirmed the observations made by Harper *et al.* (1979) that ingestion of numerous dietary compounds has measurable effects on blood.

It has been established in this study that feeding direct FMOSM at high dietary levels above 10% will elicit poor performance, cause aberrations in some biochemical indices and affect haematological parameters adversely.

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Table 3b: Enzyme activities influenced by graded levels of dietary FMOSM

Diets Enzyme (IU/L)	1	2	3	4	5
ALP	22.00[a]	27.00[b]	32.00[c]	39.00[d]	44.00[e]
AST	4.50[a]	6.00[a]	11.00[b]	15.00[c]	19.00[d]
ALT	2.67[a]	5.00[a]	9.00[b]	11.67[b]	14.00[b]
Lac. Dehydrog.	30.00[a]	37.00[b]	41.67[c]	46.67[c]	51.00[d]
Creat.kinase	16.33[a]	20.67[b]	25.00[c]	28.00[cd]	31.00[cd]

Table 4: Effects of feeding direct FMOSM on haematological parameters and their metabolites

Diets Indices	1	2	3	4	5
PCV (%)	23.00[b]	21.00[b]	20.00[b]	18.00[a]	18.00[a]
WBC x10^9/l)	36.13[c]	33.20[c]	17.73[b]	15.30[a]	12.10[a]
RBC (x10^{12}/l)	2.40[b]	2.37[a]	2.27[a]	2.27[a]	2.27[a]
Hb (g/dl)	7.87[b]	7.23[b]	6.87[a]	6.20[a]	6,20[a]
Platelet (x10^9/l)	660[c]	530[b]	520[b]	516[b]	483[a]
MCV (fl)	103.00[c]	90.00[b]	86.00[b]	81.00[a]	80.00[a]
MCH (pg)	35.00[b]	30.00[a]	29.00[a]	28.00[a]	27.00[a]
MCHC (g/dl)	34.00[b]	34.00[b]	33.00[a]	33.00[a]	33.00[a]
Total bilirubin (mmol/l)	3.00[a]	3.33[a]	4.00[b]	4.67[b]	5.33[c]
Direct bilirubin (mmol/l)	0.17[a]	0.20[b]	0.20[b]	0.20[b]	0.20[b]

DISCUSSION

Feed consumption decreased following inclusion of FMOSM in diets at graded levels in comparison with the conventional diet probably due to the increased nutrient density of the moringa based diets operating in concert with high metabolizable energy content since the seed meal used was fed in full-fat form which might have exhibited extra-caloric effect in the birds relative to the intake on the control diet since high fat/oil-based diets impart more energy in diets than same unit of carbohydrate. Reduction in feed intake following dietary increase in level of moringa seed meal could also be attributable to bitter taste of the meal known to contain the bitter chemical, saponin. Body weight gain and efficiency of feed utilization followed similar trend as that on feed intake since values on the control diet were superior to those on the test diets which became inferior as the dietary inclusion of FMOSM increased from 7.5 to 15%. Result on performance indices obtained in this study is in agreement with past reports (Ash *et al.*, 1992, Chand *et al.*, 2006, Olugbemi et al. 2010; Abbas and Ahmed, 2012) stated that inclusion of moringa products in poultry diet exceeding 10% led to depression in performance characteristics. Survival rate on both the conventional and test diets was 100%