Wayamba Journal of Animal Science – ISSN: 2012-578X; P832-P838, 2014
First Submitted January 06, 2014; Number 1389011505

SEED PROTEIN AND MINERALS UTILIZATION FROM MORINGA: TOXICOLOGICAL AND PHYSIOLOGICAL IMPLICATIONS OF PHYTO-CHEMICALS AND HEAVY METALS IN FED BROILERS

Annongu, A. A[1]., Belewu, M. A[1]., Karim, O.R.[2], Adeyina, O. A[1]., Kayode, R. M. O[2*]. Sola-Ojo, F. E[1]. and Aremu, J. O[3]
[1] Unit of Nutritional Biochemistry & Toxicology, Department of Animal Production, University of Ilorin, Nigeria., [2] Department of Home Economics and Food Science, University of Ilorin, Nigeria. [3] Livestock Department Kwara State Agricultural Development Project, Ilorin, Nigeria.

**Corresponding author:- kayodermosnr@gmail.com*

Investigations on the utilization of nutrients from full-fat *Moringa oleifera* seed meal (FMOSM) and the toxicological and physiological implications of the seeds phytochemicals and heavy metals on utilization of the nutrients were conducted. Dietary FMOSM was offered to broiler chicks at hatch for 4-weeks in a one-way classification design experiment. Response criteria from the birds were determined on nutrients utilization, bioavailability of minerals including heavy metals and effects on blood constituents and metabolites. FMOSM was included in diets at graded levels of 0.00, 7.50, 10.00, 12.50 and 15.00% for diets 1, 2, 3, 4 and 5 respectively. Increasing level of virgin seed meal in diets caused a concomitant decrease in almost all the indices measured ($p < 0.05$), probably due to the anti-nutrients/toxic factors in the unprocessed seed meal. The birds utilized the rich minerals content of *M. oleifera* including its heavy metals, which prolonged consumption might be inimical to the health status of the experimental animal models. It was therefore concluded that untreated full-fat seed meal be included in diets for poultry not beyond 10% for optimum results.

Keywords: *Moringa oleifera* seeds, nutrients, broiler chicks, anti-nutrients/toxins, heavy metals.

Moringa oleifera known locally as Jeelegede, Eweigbale, Ikwa oyibo, Zogale or Horseradish tree in Nigerian dialects is a perennial tree presently cultivated in semi-arid, tropical and subtropical countries of the world due to its nutritional, therapeutic and prophylactic properties (Fahey, 2005; Wikipedia, 2009). Nearly all parts of the tree are edible and used for food and feed (Ram, 1994). The Trees for Life Organization (2005) reported the nutritional attributes of *M. oleifera* that 28.35g leaves for instance, contain protein which quality rivals that of eggs and milk, more calcium, iron, potassium, ascorbic acid than milk, spinach, bananas, carrots and oranges respectively.

Protein and minerals are vital nutrients in man and animal bodies since protein nutrients are used for growth and tissue repair, synthesis of hormones and enzymes while minerals are essential for osmotic regulation, activation of enzymes, hormones and other organic molecules that enhance growth, functions and maintenance of life processes (Aslam et al. 2005). Past works (Rajurkar and Damame, 1998, Choudhary and Rehman, 2002; Al-Khaarusi et al. 2009) noted that the mineral composition of a plant plays significant role in its nutritional, medicinal and therapeutic values. The phytochemistry of *M. oleifera* reveals that the tree and the other related 13- species contain organic compoubds including lectins, alkaloids, glucosinolates, saponins, nitrate, oxalates, isothiocyanates, phytates (Duke, 1983; Radovich, 2011, Fahey et al.2001; Bennett et al. 2003; Makkar and Becker, 1997). Moringa is also implicated in the storage of many heavy metals which are either beneficial or toxic when present in certain concentrations. The objective of this study was to investigate the direct inclusion of full-fat seed meal at graded levels in diets and evaluate the toxicological and physiological effects of the phytotoxins and heavy metals on the fed animal models.

MATERIALS AND METHODS

Died, sorted seeds of *M. oleifera* were obtained from a plantation in northern Nigeria. 10kg viable seeds were pounded into meal using mortar and pestle followed by sieving to get desired particle size for the day-old chicks. The full-fat *Moringa oleifera* seed meal (FMOSM) was used in formulating and mixing of diets for the experimental birds. Five diets with comparable energy and protein contents were formulated made of a corn-soy reference diet and the other diets containing on as fed basis 7.50, 10.00 12.50 and 15% FMOSM for diets 2, 3,4 and 5 respectively. One hundred and twenty day-old broiler chicks from a commercial source were used for the trial and brooded in an electrically heated battery brooder cage partitioned into units for replication. Chicks were allotted at random to the 5-dietary treatments in a single-factor design model. They were fed the experimental diets twice daily, 8.00am and 2.00pm NT. Feeding was to appetite with generous supply of drinking water during a trial that lasted 4-weeks. The composition of the experimental diets and their analyzed nutrients content are shown on Table 1. Half way into the experiment, a nutrient retention trial was conducted. Weighed amount of feed was fed for 72hrs and feces voided for the 3-days were collected and dried to constant weight and milled for the determination of nutrient retention parameters. Related bio-data to the nutrient retention such as net protein utilization (NPU), nitrogen metabolism (NM), protein efficiency ratio (PER) and protein replacement value (PRV) were also evaluated.

At the end of the experiment, one broiler per replicate from each dietary treatment was randomly taken and slaughtered for the collection of blood for haematological, serum chemistry analysis and for skeletal (femorar) removal to determine bone mineralization. Haematological specimens were collected in EDTA-anticoagulant treated bottles while blood for serum chemistry analysis was collected in sample bottles without the anticoagulant. Femoral bones as representation of the entire skeleton were separated by treating the broilers with boiled water to loosen the muscles. The bone samples were oven-dried and milled for the determination of macro- and micro-minerals including heavy metals.

Chemical analysis

Analysis of the nutrients content of the diets, residual nutrients in the fecal samples and carcass protein content were determined by the methods of AOAC (2005). Bone samples were wet-digested with a mixture of nitric, sulphuric and perchloric acids before analyzing for minerals using Atomic absorption spectrophotometer (UNICAM 929 Model, UK). Phosphorus was determined by the phosphomolybdeona-vanadate method (AOAC, 1980). Haematological indices, packed cell volume (PCV), red blood cell (RBC), white blood cell (WBC) and haemoglobin (Hb) were determined using automated haematological analyzer while serum chemistry was analyzed by using commercial kit.

Statistical analysis

All data collected were analyzed following a single-factor design analysis of variance and differences between treatment means separated using Duncan's multiple range tests (Steel and Torrie, 1980).

RESULTS

Data on nutrient retention and related bio-data influenced by dietary inclusion levels of FMOSM is presented on Table 2. Ingestion of dietary FMOSM at graded levels decreased protein and soluble carbohydrate retention ($p < 0.05$) while increasing the retention of dry organic matter, fat, fibre and mineral matter relative to the reference diet (< 0.05). Apparent nitrogen intake, daily retained nitrogen and nitrogen coefficient followed the pattern similar to that on protein retention as there was significant decrease in these values at higher inclusion level(s) of FMOSM in diets compared with the conventional diet ($p < 0.05$). Apparent gross energy intake and daily absorbed energy were higher at high inclusion levels of FMOSM in diets when compared with the standard diet ($p < 0.05$) however, energy metabolism (ME) was depressed following inclusion of the virgin seed meal at the highest inclusion of 15%. Consumption of the untreated seed meal in diets depleted NPU, PER, PRV and NM relative to the control diet ($p < 0.05$).

Table 3 gives data on bioavailability of macro-, micro-minerals and composition of heavy metals in broilers receiving unprocessed moringa seed meal in diets. Availability of the minerals was observed to diminish with increasing level of the raw seed meal compared with the orthodox diet ($p < 0.05$) except for the mineral, selenium which concentration increased with increasing levels of the FMOSM in diets. The concentrations of heavy metals in the seeds was at the high side ($p < 0.05$). While nickel (Ni), copper (Cu), and cadmium (Cd) were decreasing in amount with increasing level of the seed meal in diets, lead (Pb) and vanadium (V) increased in concentration in response to higher inclusion of the full-fat

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

Diets	1	2	3	4	5
Ingredients					
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
Vit-premix	0.50	0.50	0.50	0.50	0.50
Total	100.00	100.00	100.00	100.00	100.00

Table 2: Dietary influence of graded levels of FMOSM on nutrient retention/utilization

Diets	1	2	3	4	5
Parameters					
DOM	75.00^{a}	90.00^{b}	93.33^{b}	93.00^{b}	93.67^{b}
Protein(%)	28.43^{b}	27.27^{b}	23.61^{a}	22.02^{a}	20.99^{a}
Fat (%)	8.38^{a}	27.38^{b}	26.89^{b}	26.79^{b}	24.00^{b}
Fibre (%)	12.38^{a}	19.42^{c}	24.04^{b}	25.50^{b}	26.84^{b}
Total ash (%)	10.39^{a}	11.46^{a}	20.85^{b}	30.17c	35.45^{d}
NFE (%)	34.21^{b}	8.67^{a}	8.60^{a}	6.59^{a}	4.71^{a}
AGEI (kcal/g)	7.83^{b}	4.27^{a}	4.63^{a}	11.92^{c}	22.25^{d}
DAE (kcal/g)	0.021^{a}	0.027^{b}	0.027^{b}	0.026^{b}	0.024^{b}
ME (kcal/g)	22.97^{b}	36.88^{d}	25.48^{c}	21.36^{b}	17.10^{a}
ANI (%)	3.60^{b}	2.94^{a}	3.04^{a}	3.25a	3.08a
NC	0.25b	0.45d	0.38c	0.27^{b}	0.21^{a}
DRN (%)	0.021^{b}	0.019^{a}	0.018^{a}	0.018^{a}	0.018^{a}
NPU	373^{a}	287^{b}	293^{c}	381^{d}	343^{e}
NM	96.53^{d}	37.30^{c}	26.00^{b}	18.87^{a}	18.18^{a}
PER	15.65^{b}	7.40^{a}	4.96^{a}	3.91^{a}	3.26^{a}
PRV	0.00^{a}	0.18^{d}	0.16^{d}	0.14^{c}	0.10^{b}

a-e mean values in rows not sharing common letters differed significantly ($p < 0.05$).
DOM, dry organic matter; AGEI, apparent gross energy intake; DAE, daily absorbed energy; ANI, apparent nitrogen intake; NC, nitrogen coefficient; DRN, daily retained nitrogen.

seed meal in diets ($p < 0.05$).
Haematological results (table 4)in this study serum of broilers offered FMOSM based diets ($p < 0.05$).

Table 3; Bioavailability of macro-, micro-elements and heavy metals in broilers fed dietary FMOSM

Diets	1	2	3	5	5
Elements					
Sodium (%)	0.14[c]	0.13[b]	0.13[b]	0.12[a]	0.12[a]
Potassium (%)	0.02	0.02	0.019	0.018	0.017 NS
Phosphorus (%)	18.17[c]	17.94[b]	17.63[b]	16.97[a]	16.80[a]
Calcium (%)	36.14[d]	35.61[b]	34.18[c]	34.03a[b]	33.94[a]
Magnesium (%)	0.71	0.69	0.69	0.67	0.64 NS
Manganese (%)	7.14[c]	6.88[b]	6.25[b]	5.91[a]	5.14[a]
Iron (ppm)	548	587	618	652	691NS
Zinc (ppm)	2107[c]	2058[b]	1964[a]	1946[a]	1924[a]
Selenium (ppm)	0.06[a]	0.07[b]	0.07[b]	0.072[b]	0.08[c]
Lead (mg/kg)	0.11[c]	0.10[a]	0.10[a]	0.12[b]	0.22[d]
Copper (mg/kg)	743[b]	725[b]	710[b]	626[a]	665[a]
Nickel (mg/kg)	12.66[d]	9.98[c]	10.17[c]	7.14[a]	8.20[b]
Cadmium (mg/kg)	0.009[e]	0.005[a]	0.006[b]	0.007[c]	0.008[d]
Vanadium (mg/kg)	0.0006[a]	0.0006[a]	0.0006[a]	0.0007[b]	0.0008[c]
Cobalt (mg/kg)	3.10[c]	0.85[a]	0.96[a]	2.14[b]	2.66[b]
Chronium (mg/kg)	12.66[d]	7.14[a]	8.20[b]	9.98[c]	10.17[c]

Table 4: Effects of dietary graded levels of FMOSM on some haematological parameters and metabolites

Diets	1	2	3	4	5
Indices					
PCV (%)	23.00[b]	21.00[b]	20.00[b]	18.00[a]	18.00[a]
WBC (109/L)	36.13[c]	33.20[c]	17.73[b]	15.30[a]	12.10[a]
RBC (x1012/L	2.40[b]	2.37[b]	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]
Platelets (x109/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 (%)	3.00[a]	3.33[a]	4.00[b]	4.67[b]	5.33[c]
Direct bilirubin (%)	0.17[a]	0.20[b]	0.20[b]	0.20[b]	0.20[b]

indicated that PCV, WBC, RBC, Hb, platelets as well as MCV, MCH and MCHC of broilers maintained on raw FMOSM based diets presented significantly different values from the conventional diet ($p < 0.05$). The blood parameters decreased concomitantly with increasing level of dietary FMOSM. Similarly, there was statistical significant increase in values of total and direct (conjugated) bilirubin in

DISCUSSION

Consumption of dietary virgin moringa seed meal at graded levels in diets decreased retention of nutrients, protein, fibre, and soluble carbohydrate especially at higher inclusion level (15%). The utilization of nutrients expressed as daily retained nitrogen, absorbed energy and metabolizable energy, nitrogen coefficient depicted significant decrease in values with increasing level of FMOSM in diets. Similarly, related data on NM, PER and PRV presented decrease in values in response to increasing dietary levels of the seed meal. Findings on nutrients utilization agreed with past works (Elegbede, 1998; Prohp et al. 2004) which reported that feed materials containing biologically active principles or anti-nutritional factors such as those present in *M. oleifera* have adverse nutritional and physiological impact on the consumer. Earlier, Ngoddy (1985) established that intake of dietary active principles interfere with digestive processes thereby preventing efficient utilization of nutrients and could precipitate deleterious effects in the fed man or animal.

Consideration of bioavailability of macro-elements and micro-minerals in the fed broilers vis-à-vis the anti-nutrients content of the test feedstuff indicated that the birds were able to utilize the moringa minerals especially at inclusion levels less than 15% except for selenium. Aletor and Adeogun (1995) noted that the nutritional significance of a food/feedstuff depends on its nutrients and anti-nutritional constituents. *M. oleifera* , though rich in all the essential nutrients, is implicated in the possession of noxious chemical compounds namely polyphenols, phytates, oxalates (Makkar and Becker, 1997). Phytates and oxalates are anti-metals which bind or complex especially bivalent minerals, Ca_{2+}. Mg^{2+}, Fe^{2+}, Fe^{2+} making them unavailable to the body particularly in monogastrics. This may explain why decreases were observed in mineral content in the group of birds fed the test feedstuff in diets at increasing levels. Concentrations of the heavy metals in the moringa based diets increased with increasing level of the dietary seed meal. Heavy metals such as zinc, iron, copper, chromium are beneficial in the body only when present in trace amounts required by the body. However, metals like Ni, As, Cd, Hg, Pb seem to have no beneficial effects at low or high amount. In this experiment, most of the heavy metals which contents were determined in the body of the animal models offered dietary FMOSM at graded levels were above the maximum allowable limit of WHO (2003). High value of iron above the accepted limit (0.1)and copper as recorded in this study following prolonged intake may lead to liver disease of haermosiderosis and chronic anaemia respectively in the fed birds (Rajappa et al. 2010; Acharya et al. 2008). Lead (Pb) is the most significant of all the heavy metals because it is very toxic even in small amounts (Gregoriaadou et al. 2001). Lead is chemically similar to cadmium hence their concentration in the body particularly, lead can cause hyper-excitability, delirium, convulsion and progressive lethargy leading to coma and death or permanent damage to the central nervous system including brain and kidneys (Hanaa et al. 2000).

Evaluation of the effect of dietary graded levels of FMOSM on some haematological parameters and metabolites showed that ingestion of the dietary seed meal resulted to a corresponding decrease in all the measured blood indices as the inclusion level of the test feedstuff in diets increased. Conversely, total bilirubin presented elevated values in response to increasing dietary FMOSM. Findings blood composition and metabolite confirmed the report of Harper et al. (19790 who documented that ingestion of numerous dietary compounds/substances has measurable effects on blood constituents. Since blood plays a vital role in the physiological, nutritional and pathological status of an organism, decrease in blood fraction counts observed in this study could be inimical to the health status of the experimental animal models in question.

In summary, feeding full-fat unprocessed *M. oleifera* seed meal in diets at levels above 10% could elicit deleterious effects on the fed animal.

REFERENCES

1. Acharya, G. D., Hathi, M. V., Pater, A. D. and Parmar, K. C. (2008). Chemical properties of ground water in Bailoda laluka region, north Gujarat, India, Reviewed 23 June,2010.
2. Aletor, V. A. and O. A. Adeogun (19950. Nutrient and anti-nutrients components of some tropical leafy vegetables. Food Chem. J., 53: 375-379.

3. Aletor, V. A. and O. A. Omodara (1994). Studies on some leguminous browse plants with particular reference to their proximate, mineral and some endogenous anti-nutritional constituents. Anim. Feed Sci. Tec., 46: 343-348.
4. Al-Kharusi, L. M., M. O. Elmardi, A. Ali, F. A. J. Al=Said, K. M. Abdelbasit and S. Al-Rawahi (2009). Effect of mineral and organic fertilizers on the chemical characteristics and quality of Date fruits. Int. J. Agric. Biol., 11: 290-296.
5. AOAC (2005). Official Method of Analysis, 19th Edn., Washington D.C., Association of Official Analytical Chemists.Steel, R. G. D. and Torrie, J. H. (1980). McGraw Hill Book Coo. Inc. New York.
6. Aslam, M. F., Anuar, R., Nadeem, U., Rashid, T. G., Kazi and Nadeem, M. (2005). Mineral composition of Moringa oleifera leaves and pods from different regions of Punjab, Pakistan. Asian J. Plant Sci., 4: 417-421.
7. Bennett, R. N., Mellon, F. A., Foidl, N., Pratt, J. H., DuPont, M. S., Perkins, L. and Kroon, P. A. (2003). Profiling glucosinolates and phenolics in vegetative and reproductive tissues of the multipurpose tree , Moringa oleifera L. (Horseradish tree) and Moringa stenopetala L. Journal of Agricultural and Food Chemistry, 51: 3546-3553.
8. Choudhary, M. I. and A. Rehman (2002). Recent Discoveries in Natural Products Chemistry, 7th EURASS, p: 25. A conference on chemical sciences, HEJ-Res. Ins. Chem., university of Kerachi, Pakistan.
9. Duke, J. A. (1983). Hand book of energy crop. Canada, Pp 66-69.
10. Elegbe, J. A. (1998). Legumes. In: Nutritional Quality of Plant Foods. (Ed) Ambik Press, Benin city Nig., 53-83.
11. Fahey, J. W. (2005). Moringa oleifera: A review of the medicinalevidence for its nutritional, therapeutic and prophylactic properties. Part I. Trees for Life J. 1(5): 1-19.
12. Fahey, J. W., A. T. Zalcmann and P. Talalay (2001). The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. Phytochemistry 56(1): 5-51.
13. Gregoriadou, A., Delidou, K., Dermosonoglou, D., Tsoumparis, P., Edipidi, C and Katsougiannopoulos, B. (2001). Heavy metals in drinking water in Thessaloniki area, Greece. In: Proceedings of the 7th Int. Conf. on Environmental science & Tech., Aristotle University, Ermoupolis, Greece.
14. Hanaa, M., Eweida, A. and Farag, A. (2000). Heavy metals in drinking water and their environmental impact on human health. Int. Conf. on Environ. Hazards Mitigation, Cairo University, pp 542=556.
15. Harper, A. E., Rowel, V. W. and Mayas, P. A. (1979). Review of Physiological Chemistry, 11th Edn, Lang Medical, Los Altos, California 94221 60-81-188-216.
16. Makkar, H. P. S. and K. Becker (1997). Nutrients and anti-quality factors in different morphological parts of the Moringa oleifera tree. Journal of Agricultural science 128 (3): 311-322.
17. Prohp, T. P., E. A. Mende, A. O. Madusha, S.C. Uzearu, A. Aigbiremolen and P. C. Onyebuagu (2004). Cyanide contents of Pride of Barbados (Caesalpina pulcherrima) grown in different parts of Nigeria. J. Med. Lab. Sci. 13: 29-32.
18. Radovich, T. (2011). Farm and forestry production and marketing profile for Moringa (Moringa oleifera) In: Elevitch, C. R. (ed). Specialty crops for Pacific Island Aroforestry. Permanent agriculture resources (PAR), Honolulu, Hawaii htt://agroforestry.net/scps.
19. Rajappa, B., Manjappa, S. and Puttaiah, E. T. (2010). Monitoring of

heavy metal concentration in ground water of Hakinaka Taluk. India Contemporary Engineering sciences, 2(4): 183-190.

20. Rajurkar, N. S. and M. M. Damame (1998). Mineral content of Medicinal Plants used in Treatment of diseases resulting from urinary tract disorders. Biol. Trace Elem. Res., 65: 251-259.
21. Ram, J. 91994). Moringa: A Highly Nutritious Vegetable Tree. Tropical Rural and Island/Atoll Development, Experimental Station (TRIADES), Technical Bulletin No 2.
22. Trees for Life (2005). Moringa Book htt://www.treesforlife.org/ project/ moringa/book/ default.asp.
23. WHO (2003). World Health Organization: guideline for drinking water quality, Geneva WHO/SDE/WSH 03. 04.
24. Wikipaedia (2009). Moringa oleifera. Wikipaedia Foundation, Inc. http://en.Wikipedia.org/wiki/Moringa_oleifera.

