International Journal of Advanced Scientific Discoveries

MSIS

Article

IRRADIATION DEACTIVATION OF ANTI NUTRITIONAL FACTORS IN MORINGA OLEIFERA SEEDS FOR MEAT CHICKEN

Firstname Lastname [1], Firstname Lastname [2] and Firstname Lastname [2,*]

[1] Azor Amos Annongu
[2] Jimoh Olalekan Aremu
[2] David Friday Apata
[3] Johannes Hughes Edoh
[4] Jimo Ige Sanni
[5] Adedayo Olugbenga Gawati
[6] Zainab Bello-Bukoye
[7] Ololade Saheedat Abdussalam
* Correspondence: jimoh4olalekan@gmail.com; Tel.: +2348038541125)

Abstract

Phytochemical composition and deactivation of anti-nutritional factors in *Moringa oleifera* seeds (MOS) to replace soybeans in diet for chicks were investigated. 5-experimental diets were formulated and fed to 180 -Arbor Acre broiler chicks at hatch over a feeding trial lasting 56-days. Each dietary treatment contained 3-replicates of 36-chicks. The diets were made of a control diet1 (free of MOS), a negative control diet 2 containing 5% MOS and 3 other diets 3, 4 and 5 containing 5% gamma-irradiated *Moringa oleifera* Seed Meal (GIMOSM) at graded doses of 10, 30 and 50kGy respectively. The experiment followed a one -way classification design. Chemical analysis of the raw seed gave valuable amounts of nutrients as well as anti-nutrients which presence could elicit health hazards when consumed in diet in certain amount. Results on performance traits, nutrient digestibility (crude protein, fat and fibre) gave poor results in the negative/raw MOS control diet ($p < 0.05$) compared to the control and GIMOSM diets. GIMOSM diets improved ($p < 0.05$) haematological (PCV, RBC, HB) and biochemical (urea, AST, ALT, ALP) parameters measured in broiler nutrition. Findings of this study showed that the detrimental effect induce by the anti-nutrients present in the raw MOS on the measured biological parameters were reversed to good by g-irradiation treatment. It is suggested that MOS meal be treated at 10kGy level to safely detoxify its anti-nutritional and/or toxic factors for optimum use in poultry nutrition.

Key words: MOS, chemicals, gamma-irradiation, haematological-biochemical indexes.

1. Introduction

The prohibitive cost of conventional oilseeds which supply protein-rich residue as cake after oil extraction that can be used as protein supplements in animal diet necessitate research on non-conventional ingredients such as *Moringa oleifera* seeds. For this reason, continuous search is ongoing in developing countries for lesser known or novel or inexpensive but high nutritive feed ingredients like *Moringa oleifera* seeds which have high potential as important protein supplements for feeding livestock especially monogastric animals which share similar feeding materials with human beings. These feeding stuffs are competed for by man, animals and industries (1). Though considered as a wonder tree due to its products in terms of their nutritive, medicinal and industrial values, *M. oleifera* is reported to contain anti-nutritional factors that can impact or elicit toxic response in the fed subject due to the presence of the toxic factors (2,3). Phytochemicals can be primary or secondary metabolites in nature. Phytochemicals termed "secondary metabolites" can be useful or poisonous when consumed depending on level of intake (4-7). Many processing techniques abound on anti-nutritional factors in human and animal foods/feeds to improve their nutritional values for efficient maximization of their use in nutrition. Past works reported that some anti-nutritional factors could significantly be inactivated by ionizing gamma irradiation (8-11). Foods/feeds treatment by irradiation is a relatively new processing technique developed in recent time in the course of technological advancement.

Since discovery of the method, irradiation treatment has been applied in many areas including agriculture. Processing of feeding materials by this technique involves ionization with radioactive particles from a particular radioisotope like cobalt-60, caesium-137 or use of accelerated electrons from an accelerator with energies up to 10MeV or use of X-rays with energies up to 5MeV [12]. The objective of this study, therefore, is to attempt irradiation detoxification of antinutritional factors in *M. oleifera* seed by gamma irradiation as an alternative protein source in broiler chicks' nutrition.

2. Experiments and Methods

2.1. Study Area and Experimental Design

The poultry unit of the Teaching and Research Farm, Kwara State University, Malete, Nigeria was used as an experimental site for the feeding trial. *M. oleifera* seed cake was obtained from Moringa Plantation at Afe Babalola University, Ado-Ekiti, Ekiti State of Nigeria. The cake was properly sundried and pulverized into flour to meet the particle size requirement of day-old chicks in diets mixtures [13]. 15kg flour was divided into four equal batches and placed in open polyethylene bags. Three of the bags with the flour were irradiated at once in a gamma irradiating machine (model number L-110) until the respective total doses for 10, 30 and 50kGy were achieved. The fourth batch was not irradiated representing untreated *M. oleifera* seed flour.

2.2. Diets formulation

Five experimental diets were formulated made of a control diet without Moringa seed meal (diet1) and four other diets out of which raw/untreated Moringa seed meal for diet 2 was made as the negative control. Diets 3, 4 and 5 contained gamma-irradiated *Moringa oleifera* seed meal (GIMOSM) at graded doses of 10, 30 and 50kGy respectively.

M. oleifera seed meal substituted soyabean meal in diets at 5% inclusion level. The composition of the experimental diets on as fed basis including the calculated nutrient contents of the diets are shown in tables 1a and b

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

Diets	**1**	**2**	**3**	**4**	**5**
Radiat. Doses (kGy)	**0**	**0**	**10**	**30**	**50**
Ingredients					
Maize	52.00	52.00	52.00	52.00	52.00
GIMOSM	0.00	5.00	5.00	5.00	5.00
Soybean meal	31.00	26.00	26.00	26.00	26.00
Wheat offal	10.00	10.00	10.00	10.00	10.00
Palm oil	1.50	1.50	1.50	1.50	1.50
Lysine	0.10	0.10	0.10	0.10	0.10
Methionine	0.25	0.25	0.25	0.25	0.25
Bone meal	1.15	1.15	1.15	1.15	1.15

Oyster shell	1.50	1.50	1.50	1.50	1.50
Salt	0.25	0.25	0.25	0.25	0.25
Vit/min premix	0.25	0.25	0.25	0.25	0.25
Fish meal	2.00	2.00	2.00	2.00	2.00
Total	**100**	**100**	**100**	**100**	**100**

Table 1b: Calculated nutrients content of diets.

Diets	**1**	**2**	**3**	**4**	**5**
Chemicals/diets					
ME (Kcal/kg)	2954	2959	2943	2958	2946
Crude protein,%	23	23	23	23	23
Crude fibre,%	3.20	4.11	3.13	3.58	4.23
Crude fat,%	4.65	9.13	5.85	6.23	4.67
Methionin,%	0.49	0.58	0.35	0.48	0.45
Lysine, %	1.25	1.50	1.10	1.45	1.10

2.3. Test animals and feeding trial

One hundred and eighty Arbor Acre broiler chicks at hatch were used for the trial. The experiment was designed as a one-way classification. Each of the five dietary treatments contained three replicates with twelve (12) chicks per replicate. The chicks were fed on a conventional diet for a week to acclimatize them to the environment prior to introduction of the experimental diets.

The birds were thereafter fed the experimental diets ad libitum during a feeding trial that lasted 56-days.

2.4. Nutrients Digestibility trial

After the feeding trial, a digestibility study was performed using INRA reference protocol [14]. Fifteen broiler chickens were taken on all the dietary treatments (3-per diet) and placed in individual metabolic cage designed for the digestibility study. The birds, similar in weight ($p>0.05$) were acclimatized for three-days, before fasting for 24 hours. 200g of the corresponding diet was given to each bird for 3 days followed by another day of fasting. Excreta was collected from the replicates on all the diets during the three days of feeding and the last day of fasting. The quantity of feed served, and the ort (leftover) were weighed. Proximate analysis was carried out on the diets and the fecal samples for the calculation of nutrient digestibility or nutrient retention. Nutrient digestibility was calculated using the formula:

$$\%\ \text{Nutrient digestibility} = \text{Nutrient intake - Nutrient in excreta} / \text{Nutrient intake} \times 100.$$

2.5 Chemical analysis

The nutritional composition of raw *M. oleifera* milled seeds, the macro and micro-mineral contents of the untreated seed, the phytochemical constituents and nutrients digestibility analysis were carried out in the Department of Chemistry laboratories, University of Ilorin while gamma-irradiation of the seed flour for treated *M. oleifera* seed meal was carried out at the commercial laboratory, Central Research and Diagnostic Laboratory, Tanke, Ilorin, Nigeria. Qualitative screening and quantification analyses were carried out for *M. oleifera* phytochemicals, saponins, anti-metals (oxalates and phytates), polyphenols including tannins, alkaloids, flavonoids, glycosides, terpenoids using the procedures of past workers

[15,16] The analytical methods of AOAC [17] were used for determination of mineral composition.

2.6. Statistical analysis

Data collected on chemical analysis were presented by descriptive statistics while data on performance, nutrient digestibility, haematological and biochemical indices were analyzed using the analysis of variance (ANOVA) according to the single factor design model. Differences between treatment means were separated using Duncan multiple range test as described by Steel and Torrie [18].

3. Results and Discussion

3.1. Chemical composition

Nutritional (proximate) composition of raw *M. oleifera* seeds and its mineral composition are presented in tables 2. The seed is rich in nutrients, dry matter, crude protein, fat, mineral matter as well as nutrient minerals such as phosphorus, magnesium, manganese, iron, zinc.

Table 2a: Proximate composition of raw Moringa oleifera milled seed cake (MOMSC)

Indexes	Percent Levels
Dry matter	89.31
Crude protein	33.82
Ether extract	13.37
Mineral matter	4.74
Crude fibre	5.10
Carbohydrates	32.30

Table 2b: Mineral constituents of virgin MOMSC

Mineral	Level (mg/100g sample)
Magnesium	84.60
Phosphorus	634.01
Aluminium	0.09
Boron	13.60
Cobalt	0.18
Manganese	85.32
Molybdenum	125.69
Iron	51.80
Nickel	3.20
Copper	2.86
Selenium	0.58
Zinc	0.08
Chromium	< 0.01

Cadmium	< 0.01
Arsenic	0.00
Lead	0.00

3.2.The Phytochemicals

The phytochemical constituents of the seed flour are presented in table 3. There are notable phytochemicals in the seed such as saponins, phytates, tannins, oxalates, alkaloids, flavonoids, phenols, glycosides and terpenoids

Table 3: Phytochemical composition of raw MOSMC

Chemical	Amount
Saponins (%)	36.10
Phytates (mg/100g)	173.20
Tannins (mg/100g)	132.16
Oxalates (mg/100g)	109.40
Alkaloids (mg/100g)	283.58
Flavonoids (%)	86.42
Phenols	38.16
Glycosides (%)	0.00
Terpenoids (%)	72.44

3.3. Growth performance

Growth performance of broiler chickens fed gamma-irradiated MOSM based diets is shown in table 4. Feed intake on milled raw seeds in diet (negative control) was low compared with the conventional/control diet and diets containing MOSM treated by irradiation. Daily body weight gain and feed conversion efficiency followed similar trend as feed intake

Table 4: Comparative performance of broiler chickens fed gamma-irradiated *Moringa oleifera* Seed Meal (GIMOSM) diets relative to the control and raw milled seed diets.

Diets	**1**	**2**	**3**	**4**	**5**	**±SEM**
Irradiation MOSM Dose (kGy)	**0(control)**	**0(raw)**	**10**	**30**	**50**	
Parameter						
Feed intake(g/b/d)	104^{a}	98^{b}	106^{a}	108^{a}	107^{a}	3.94
Weight gain(g/b/d)	59^{a}	47^{b}	59^{a}	59^{a}	58^{a}	1.99
Feed efficiency(F/G)	1.7^{b}	2.1^{a}	1.8^{b}	1.8^{b}	$1b.8^{b}$	0.05

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

3.4. Digestibility Performance

Digestibility of nutrients (crude protein, fat and fibre) in the experimental birds is presented in table 5. The digestibility of nutrients on the control diet was comparable with that of the diets containing gamma-treated MOSM ($p > 0.05$) while digestibility of the nutrients on dietary milled raw seed was inferior compared with the control diet and the gamma-treated MOSM diets ($p < 0.05$).

Table 5: Nutrient digestibility of broilers given g-irradiated MOSM based diets compared to conventional and raw MOS diets

Diets	**1**	**2**	**3**	**4**	**5**	**±SEM**
Irrad. Doses of MOSM (kGy)	**0**	**0**	**10**	**30**	**50**	
Indices (%)						
Crude protein	78^a	61^b	77^a	7^8	76^a	2.6
Crude fat	73^a	58^b	73^a	73^a	76^a	2.4
Crude fibre	66^a	52^b	65^a	66^a	64^a	2.2

3.5. Haematological and Biochemical Parameters

Haematological and biochemical parameters of chickens fed gamma-processed MOSM in diets compared with the control and diet with raw seed flour are presented in tables 6 and 7. Treatments appeared to improve blood composition indices of the packed cell volume, erythrocytes, haemoglobin relative to the diet untreated MOS ($p < 0.05$). The biochemical parameters of the protein metabolite, urea, was observed to elevate on the diet containing raw milled seeds of Moringa compared with the value of this index on the control diet and treated MOSM diets ($p < 0.05$)

Table 6: Blood composition of broilers maintained on conventional, raw and irradiated MOSM diets.

Diets	**1**	**2**	**3**	**4**	**5**	**±SEM**
Irrad. Doses of MOSM (kGy)						
Parameters						
PCV (%)	31^a	21^b	30^a	33^a	30^a	2.1
RBC(X1012/l)	2.4^a	2.1^b	2.3^a	2.5^a	2.4^a	0.2
HB (g/dl)	8.8^a	7.3^b	8.5^a	9.3^a	8.5^a	0.6
WBC(x109/l)	231	204	230	239	239 ^{NS}	6.5

MCV (fl)	132	129	132	131	133 NS	3.3
MCH (pg)	37	37	37	37	37 NS	0.66
MCHC (g/dl)	28.1	28.6	28.0	28.4	28.0 NS	0.33

NS= not significant ($p > 0.05$).

Table 7: Biochemical indices of broilers offered control, untreated and treated MOSM in diets.

Diets	**1**	**2**	**3**	**4**	**5**	**±SEM**
Irradiated doses						
Indexes						
Total protein(g/l)	14b	10a	112.6a	13b	13b	0.2
Albumin(g/l)	2.7a	3.0b	2.5a	2.8a	2.6a	0.05
ALT(IU/l)	19.5b	25a	18.9b	19b	20.0b	1.7
ALP(IU/l)	34.7b	37.3a	27.6c	26.0c	31.3b	2.5
AST(IU/l)	80.0b	96.0a	72.0c	82.0a	74,2c	3.9
Urea level(mmol/l)	4.7b	5.1a	4.4b	4.2b	4.7b	0.2

a-b-c mean averages in rows not sharin common letters are significantly different ($p < 0.05$).

4. Discussion

4.1. Nutritional (proximate) and mineral composition of raw M. oleifera seeds

The seed is observed to contain micromineral heavy metals and metalloids like nickel, copper, chromium, cadmium, molybdenum, cobalt, boron, aluminum. The availability of the essential nutrient minerals in the seed (both macro and microminerals) could be of great nutritional benefit if the available and utilization in the body are not affected adversely by presence of antinutrients. The presence of toxic heavy metals and metalloids and phytochemicals in the native seed however, explains why the seeds are harmful when fed raw in diet at certain levels [19].

4.2. Phytochemical constituents

The phytochemical constituents of the seed flour (table 3) presented some chemicals that could be beneficial in diet as well as those that can elicit harmful effects when consumed in high amounts in diet. Phytochemicals like phenolic compounds (phenolic acid, tannins, flavonoids) exhibit anti-carcinogenic, anti-mutagenic properties [20, 21]. These phenols interfere in many steps of malignant tumours, activate enzymatic systems responsible for the detoxification of xenobiotics and supply oxygen to neutralize reactive oxygen species or radicals hence serve as free radical scavenging entities that provide antioxidant effects in the body system [22-24]. Phytophenols of iso-flavones supply oestrogens and modulate oestrogen levels in human and animal bodies and are beneficial in normalizing the low oestrogen states like imbalances [25, 26]. These group of chemicals also protect against and control prostate and testicular cancers and regulation of semen quality in males, prevent breast cancer, cystic ovaries and endometriosis in female subjects [27].

On the other hand, *M. oleifera* seeds used in this study contain high antinutritional phytochemicals namely alkaloids, saponins, some terpenoids as well as anti-metals like phytates and oxalates.

High alkaloids content of the seed used in this experiment may be responsible for health hazard especially if the raw seed is ingested in diet beyond the normal threshold [2]. The presence of alkaloids and tannins in Moringa seeds is observed to make the seeds behave as nutritional inhibitors due to the combination of the toxic phytochemicals with proteins making the nutrients indigestible and unavailable to the body and causing limited feed intake when included direct in diet [3, 19] Alkaloids are also reported to cause gastrointestinal and neurological disorders while tannins act as nutritional inhibitors by decreasing feed intake, binding proteins including digestive enzymes to form complexes [1, 6, 28].

Further works by [29}and [30] reported that tannins caused decrease in palatability and reduced growth rate at high dietary intakes. The presence of harmful saponins in diet (other than the beneficial ones) have been implicated in the cause of hypocholesterolaemia by binding cholesterol making it unavailable for absorption [31, 32]. Toxic saponins have also been fingered in haemolysis of red blood cells, induction of infertility by their anti-spermal and spermicidal effects on spermatozoa due to strong damage of the spermal plasma membrane [33-36].

High phytate content as obtained in Moringa seeds used in this investigation (173.20mg/100g) could bind nutrient minerals such as calcium, iron, magnesium, zinc making the minerals unavailable [37, 38]. Similarly, anti-metals like oxalates are reported to bind divalent minerals like calcium, magnesium and to interfere with their metabolism besides causing muscular weakness, paralysis, gastrointestinal tract irritation, blockage of the renal tubules by calcium oxalate complex [39 – 41]). Previous research work by [42] indicated nephrotic lesions in the kidneys caused by high dietary oxalate intake hence oxalates and phytates constitute anti-nutritional factors eliciting toxicity when consumed in an unprocessed food/feedstuff [43, 44].

Excess soluble oxalates in the body are observed to prevent the absorption of soluble calcium ions due to binding with oxalates forming insoluble calcium-oxalate complexes leading to development of kidney stones in subjects vulnerable to kidney stones [45].

4.3. Growth performance

Performance data of broiler chickens fed gamma-irradiated MOSM based diets is shown in table 4. Feed intake on milled raw seeds in diet (negative control) was low compared with the conventional/control diet and diets containing MOSM treated by irradiation ($p < 0.05$). Low feed consumption on raw (untreated) seed flour of Moringa in diet may be due to the bitter taste imparted by the seeds due to the presence of alkaloids especially saponins [46, 47]. Raw MOS in diet also caused poor daily body weight gain and feed conversion efficiency of the diet compared with the control diet and diets with processed MOS ($p <0.05$). Daily body weight gain and feed conversion efficiency followed similar trend as feed intake. The conventional and gamma-treated seed meal diets gave comparable ($p > 0.05$) results on weight gain and feed conversion ratio (FCR). Past research works by [2; 3] reported that the presence of alkaloids, tannins in raw Moringa seeds caused the seeds to behave as nutritional inhibitors due to chelating effects of some phytochemicals with dietary nutrients like proteins, carbohydrate, minerals making these nutrients indigestible and unavailable to the body. In addition, alkaloids like saponins present in the raw seeds have been fingered in eliciting gastrointestinal and neurological disorders that exacerbated poor performance of the fed animals observed in this experiment [48].

4.4. Digestibility Performance

The digestibility of nutrients on the control diet was comparable with that of the diets containing gamma-treated MOSM ($p > 0.05$) while digestibility of the nutrients on dietary milled raw seed was inferior compared with the control diet and the gamma-treated MOSM diets ($p < 0.05$).

Result on nutrient digestibility (retention) in this study lends credence to the reports of Owens et al. [49] that the presence of alkaloids in an unprocessed feedstuff caused the feeding material to become a nutritional inhibitor to the nutrients in the feed making the nutrients indigestible and unavailable.

4.5. Haematological and Biochemical Performance

The haematological values as observed in this study indicates that treatments appeared to improve blood composition indices of the packed cell volume, erythrocytes, haemoglobin relative to the diet untreated MOS. The haematological values recorded on the test diets were comparable with those of the reference diet ($p > 0.05$). Reduction in PCV, RBC, HB counts in group of chickens fed milled raw seed in diet and improvement of their counts on the gamma-treated Moringa seed meal in diets in this study confirmed the findings of early workers [9, 10, 50 -52] that antinutritional factors are removed or significantly inactivated following gamma irradiation treatment. The absence of significant differences in white blood cell values or the derived RBC values of MCV, MCH and MCHC between the reference diet and diets containing irradiated MOSM suggests that the processing method adopted was feasible to detoxify the raw MOS antinutrients hence did not influence these parameters adversely. This result also supports the fact that treatment by gamma irradiation does not deposit residuals of radiation in the treated material or impart radioactivity in the feedstuff exposed to or treated [53, 54].

The biochemical parameters of the protein metabolite, urea, was observed to elevate on the diet containing raw milled seeds of Moringa compared with the value of this index on the control diet and treated MOSM diets ($p < 0.05$). This result connotes that the protein of raw Moringa seed in poultry diet might not be effectively utilized due to the presence of the inherent toxic and/or antinutritional factors.

Similarly, elevation in activities of the detoxifying enzymes, alanine aminotransferase, alkaline phosphatase (ALT and ALP) (table 7) in the group of birds fed the diet with untreated Moringa seed flour may could further explain the toxic nature of raw MOS and inclusion direct in diet. Although mild increase in activity of some enzymes in the system is beneficial, increments beyond the acceptable threshold level as observed on the diet containing untreated MOS in this study are inimical to the organs in which such enzymes operate [55].

Conclusion

This experiment established that detoxification of *M. oleifera* seed meal by ionizing with gamma irradiation produced an alternative protein source for use in nutrition of poultry which has no detrimental effect on nutrition and health of the fed animals. It is recommended that

Moringa oleifera milled seeds be irradiated at 10 kGy dosage to effectively eliminate or reduce the antinutritional factors in the seeds for optimal use in poultry nutrition.

Acknowledgement

The authors are grateful and thankful to the staffers of Moringa Plantation at the Afe Babalola University (ABUAD), for supplying the *M. oleifera* seed cake used in this study.

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