Journal of Clinical Trials and Case Studies

Open access

Effect of Sprouted Moringa Seed Meal on Haematological and Serum-biochemical Profiles of Growing Rabbits

*Arire EO, Raimi CO and Acheneje P

*Department of Agricultural Technology, School of Agriculture and Agricultural Technology, Federal Polytechnic, Ado-Ekiti, Ekiti State, Nigeria

RESEARCH ARTICLE

ABSTRACT

This study was conducted to evaluate the effect of graded dietary levels of sprouted moringa seed meal (SMSM) on haematological parameters and serum biochemical profiles of growing rabbits. A total of twenty four (6 weeks old) New Zealand white rabbits were randomly assigned to four dietary treatments in a completely randomized design (CRD). Each treatment was replicated thrice with two rabbits per replicate. The four dietary treatments contained basal diets supplemented with sprouted moringa seed meal; diet 1 (control) without SMSM, diet 2 (contained 5 % of SMSM), diet 3 (contained 10 % of SMSM), and diet 4 (contained 15 % of SMSM). Fresh feed and clean water were offered ad libitum twice daily throughout the period of the experiment (8 weeks). There was significant ($P < 0.05$) difference in value of packed cell volume (PCV) recorded, diet 1 (39.00 %) while diet 4 (48.00 %). Highest value of haemoglobin (16.00 g/dl) was recorded in diet 4 while the lowest value of (13.00 g/dl) was obtained in control diet. The red blood cell (RBC) values obtained in this study ranged between (3.15 - 7.38 x 106/ul). The white blood cell (WBC) value of (4.00 x 103/ul) was obtained in diet 4 while (8.9 x 103/ul) was observed in diet 3. The mean corpuscular volume (MCV) obtained was significantly ($P<0.05$) influenced by SMSM, (67.70 fl) was obtained in diet 2 which was the highest while (59.10 fl) was recorded in diet 3 which was the lowest. The mean corpuscular haemoglobin (MCH) of (4.27 - 26.67 pg) was observed in this study. The mean corpuscular haemoglobin concentration (MCHC) was not significantly ($P>0.05$) affected by the dietary treatments. Serum total protein obtained ranged from 9.29 g/dl in diet 3 to 10.39 g/dl in control diet. The serum albumin values of (6.88 - 8.21 g/dl) was recorded in this study. There was significant ($P<0.05$) difference in the value of serum globulin which ranged between (1.66-2.85 g/dl). The aspartate aminotransferase (AST) obtained revealed that highest value was observed in control diet (42.60 iu/l) while the lowest was obtained in diet 2 (25.30 iu/l). The values of alanine aminotransferase (ALT) recorded were significantly ($P<0.05$) affected, 50.70 iu/l was obtained in diet 2 while 168.00 iu/l was observed in diet 1. Highest alkaline phosphatase (ALP) value of (10.70 iu/l) was recorded in control diet while lowest value of (4.00 iu/l) was observed in diet 3. The findings of this study showed that sprouted moringa seed meal did not exert deleterious effect on blood constituents of growing rabbits.

KEYWORDS

Sprouted moringa seed, Rabbits, Haematology, Serum biochemistry, Dietary treatments

Correspondence to: Dr. Arire EO, Department of Agricultural Technology, School of Agriculture and Agricultural Technology, Federal Polytechnic, Ado-Ekiti, Ekiti State, Nigeria, Email: emman.arire@gmail.com

Received: February 27, 2024, **Accepted**: April 15, 2024, **Published**: May 12, 2024

DOI: 10.1042/JCTCS.6.2.0014

Citation: Arire EO, 2024, Effect of Sprouted Moringa Seed Meal on Haematological and Serum-biochemical Profiles of Growing Rabbits. JCTCS. Vol 6.

INTRODUCTION

High cost of livestock production is attributed to high cost of feeding. The production of rabbit cannot be effective without adequate feed and feed ingredients in the right proportion. It has been estimated that cost of feeding rabbits accounts for 70-80 % of total cost of production [1] to produce good quality feed available at reasonable price in order make rabbit production more profitable, it is imperative that necessary attention should be given to how unconventional feedstuffs that can yield optimum results in terms of quality and quantity of meat produced can be exploited so as to reduce the cost of feed and thereby make animal products affordable, thus alternative feed resources must be identified, evaluated and recommended for farmers [2]. *Moringa oleifera* seeds is an affordable alternative feedstuffs that possess medicinal properties which can be used to replace the expensive conventional feedstuffs in rabbit diet. It is a rich source of some essential nutrients such as Vitamin A and B-vitamins, calcium, iron, copper, sulfur,

amino acids, energy, crude protein and antioxidants. It is known to possess some antimicrobial properties [1]. It also has ability to absorb and neutralize toxic elements in food this justify its significance in in water purification [3]. Taiwo *et al*., [4] reported that *Moringa oleifera* seed is a good source of edible oil, protein and essential minerals. Its nutritional potentials enable it to be useful in formulations and fortifications of animal feeds. Idris and Jami [5] reported that *Moringa oleifera* is a highly valued plant and has an impressive range of medicinal uses which promotes growth through it's antimicrobial and antioxidant effects. However, it was reported to be an excellent source of proteins in monogastric animals [6]. *Moringa oleifera* was reported to contain some anti-nutritional factors, such as protease inhibitors, hemaglutinins, tannins and cyanogenic glycosides, which may interfere with the digestion and utilization of dietary protein and carbohydrates and even alters the availability of some minerals [7]. These anti-nutritional factors can be reduced by seed germination. Seed germination alter biochemical composition of the seed, during which some changes are observed in the seed which may lead to increase or reduction in quantity and type of nutrients in seed [8]. An increase of minerals, increase of protein bioavailability, and a reduction of secondary metabolites of feedstuffs are observed during germination [9]. Haematological changes are routinely used to determine various influences of environmental, nutritional and or pathological factors [10]. For proper management of rabbit, it is desirable to know the normal physiological values under normal situation. The increasing in the use of *moringa oleifera* seed as feed additive in animal feed necessitated the evaluation of the effects of graded levels of sprouted moringa seed meal on the haematological and serum-biochemical profiles of growing rabbits.

MATERIALS AND METHODS

Experimental site

The study was carried out at the rabbitry section of Teaching and Research Farm of the Agricultural Technology Department, Federal Polytechnic, Ado-Ekiti, Ekiti State, geographically located in south western zone of Nigeria. The average annual rainfall is about 1482.93 mm with minimum and maximum temperatures of 20ºC and 33 ºC and relative humidity of 50-85 %.

Experimental animals and management

A total of twenty four (6 weeks old) New Zealand white rabbits were randomly assigned to four dietary treatments in a completely randomized design (CRD). Each treatment was replicated thrice with two rabbits per replicate. Each rabbit received assigned diet for 8 weeks. Proper routine management practices were observed. Fresh feed and clean water were offered *ad libitum* twice daily throughout the duration of the experimental.

Preparation of test ingredient

Moringa seeds was purchased from a local market at Ibadan Oyo State, it was sorted and washed with clean water. The seeds were soaked in clean water for 12 h at room temperature of 32 ^{O}C (water for soaking the seeds was changed every 2 hours to prevent fermentation). After soaking, water was decanted off, the moringa seeds was drained and spread on the cemented floor and was covered with sack and left for 72 h to germinate. The germination period determined based on the results of the study of [8]. Water was sprinkled on the moringa seeds at 12-h interval to facilitate the germination process. At the end of germination, root hairs and seed coats were manually removed from the germinated seeds. Sprouted *moringa* seeds was dried at 60C in an air-dry oven followed by grinding into fine particle size. The sprouted *moringa* seed meal (SMSM) was stored in a plastic container prior to its use in rabbit feed formulation.

Experimental diets

Four experimental diets were formulated based on nutrient requirement of growing rabbit. The dietary treatments consisted of basal diets supplemented with sprouted moringa seed meal at levels of 0 (control), 5 %, 10 %, and 15 %. The diet are: diet 1 (control) without SMSM, diet 2 (contained 5 % of SMSM), diet 3 (contained 10 % of SMSM), and diet 4 (contained 15% of SM]SM). Each diet was properly grinded, mixed and pelletized. The compositions of the experimental diets is shown in (Table 1).

Ingredients	**Dietary Treatments**			
	1 (Control)	**2 (SMSM 5 %)**	**3 (SMSM 10 %)**	**4 (SMSM 15 %)**
Maize	34.5	33	31	28
Soya beans meal	23	20	18	16.5
SMSM	0	5	10	15
Palm kernel cake	9	9	9	9
Rice husk	11	11	11	11
BDG	12	12	12	12
Groundnut cake	6.5	6	5	4.5
Bone meal	2.5	2.5	2.5	2.5
Methionine	0.25	0.25	0.25	0.25

Lysine	0.25	0.25	0.25	0.25
Premix	0.5	0.5	0.5	0.5
Salt	0.5	0.5	0.5	0.5
Total	**100**	**100**	**100**	**100**
Calculated Analysis				
ME (Kcal/kg)	2399.925	2431.4	2471.449	2491.944
Crude protein (%)	19.78	19.93	20.27	20.96
Crude fibre (%)	10.13	9.9935	9.867	9.783

**Vitamin/Mineral Premix (Animal Care®) Vitamin A12000000IU, Vitamin D3 3000000IU, Vitamin E 30000mg, Vitamin K3 25000mg, Folic Acid 1000mg, Niacin 40000mg, Vitamin B2 5000mg, Vitamin B12 20mg, Vitamin B1 2000mg,Vitamin B6 3500mg, Biotin 80mg, Antoxidant 125000mg, Cobalt 250mg, Selenium 250mg, Iodine 1200mg, Iron 40000mg, Manganese 70000mg, Copper 8000mg, Zinc 60000mg Choline chloride 200000mg.*

Table 1: Composition of diet containing graded level of sprouted moringa seed meal.

DATA COLLECTION

Haematological and serum-biochemical evaluation

On the 56th day of the feeding trial blood samples were collected from the ear vein of two randomly selected rabbits per treatment for haematological and serum-biochemical profiles. The blood samples were transferred into sterile bottles containing ethyl diamine tetraacetic acid (EDTA). The blood samples were analysed for hematological parameters such as, packed cell volume (PCV), red blood cell count (RBC), haemoglobin concentration (Hb), white blood cell count (WBC) according to the methods described by Jain [11] while mean cell volume (MCV), mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC) were calculated. Blood samples for serum biochemicals evaluation were collected into other bottles without anticoagulant. The serum total protein, albumin, globulin, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were determined according to Ukorebi, [12].

STATISTICAL ANALYSES

Data were subjected to statistical analysis using statistical packaging for the social sciences (SPSS) Version 23.0 for windows. Mean separation was done using Duncan's New multiple range tests, and differences were declared significant at 5 % ($P < 0.05$).

RESULTS

Haematological Indices of Growing Rabbits fed Varying Levels of Sprouted Moringa Seed Meal Diets

The response of haematological variables of growing rabbits fed varied levels of sprouted moringa seed meal is as shown in Table 2. The haematological indices evaluated were red blood cell (RBC), haemoglobin (Hb), packed cell volume (PCV) and white blood cell (WBC), Mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC), also measured were white blood cell differentials that included lymphocytes, granulocytes and monocytes. The value of PCV and haemoglobin increase ($P<0.05$) significantly across the treatment group with increase in the inclusion level of the SMSM, this is an indication that moringa seed meal has properties that influence the blood contents of rabbit. The values of haemoglobin obtained were significantly ($P<0.05$) influenced by the experimental diets, highest value of 16.00 g/dl was recorded in diet 4 while the lowest value of 13.00 g/dl was obtained in control diet. The RBC value of 7.38 g/dl obtained in rabbits fed diet 3 was significantly ($P<0.05$) higher than value of 3.15 g/dl obtained in the rabbits on control diet. The values of WBC recorded in diet 4 (4.00 x 103/ul was significantly lower than value recorded in diet 3 (8.9 x 103/ul).

The values of MCV obtained were significantly ($P<0.05$) influenced by the experimental diets, highest value of 123.80 fl was recorded in rabbits fed diet 1 while lowest value of 59.10 fl was obtained in the rabbits fed diet 3. The MCH values of 4.27-26.67 pg was observed in this study, highest value of 26.67 pg was recorded in rabbits fed diet 4 while the lowest value of 4.27 pg was obtained in those control diet. MCHC was not significantly ($P<0.05$) affected by the dietary treatments but the highest numerical value of 33.40 g/dl was recorded in diet 3 while the lowest value of 33.25 g/dl was obtained in diet 2, The monocytes value observed ranged from 0.00 % in rabbits on diet 1 and 2 to 4.00 % in those on diet 2. All the values obtained were within the reference range of 0-4 % for healthy rabbits. The values of granulocytes obtained were significantly ($P<0.05$) influenced by the experimental diet, diet 2 had the highest value of 70.00 % while lowest value of 16.00 % was recorded in the control diet. The highest lymphocytes value of 84.00 % was obtained in rabbits fed control diet while lowest value of 26.00 % was recorded in diet 2, both the diet 3 and 4 had similar value of 40.00 %.

Parameters	Dietary Treatment				±SEM	*p*-Value
	1 (Control)	2 (SMSM 5 %)	3 (SMSM 10 %)	4 (SMSM 15 %)		
Packed cell volume (%)	39.00^{d}	43.00^{c}	44.00^{b}	48.00^{a}	0.97	0.001
Haemoglobin (g/dl)	13.00^{c}	14.30^{b}	14.70^{b}	16.00^{a}	0.35	0.001
Red blood cell (x10^{6}/ul)	3.15^{c}	6.35^{b}	7.38^{a}	6.00^{b}	0.49	0.002
White blood cell (x10^{3}/ul)	4.40^{c}	6.40^{b}	8.90^{a}	4.00^{c}	0.60	0.002
MCV (fl)	123.80^{a}	67.70^{c}	59.10^{d}	80.00^{b}	7.51	0.006
MCH (pg)	4.27^{d}	22.57^{b}	19.69^{c}	26.67^{a}	2.56	0.002
MCHC (g/dl)	33.33	33.25	33.40	33.33	0.12	0.986
Monocytes (%)	0.00^{c}	4.00^{a}	0.00^{c}	2.00^{b}	0.51	0.032
Granulocytes (%)	16.00^{d}	70.00^{a}	60.00^{b}	58.00^{c}	6.25	0.041
Lymphocytes (%)	84.00^{a}	26.00^{c}	40.00^{b}	40.00^{b}	6.58	0.013

abcd: Means with different superscripts on the same row were significantly (*P*<0.05) different. SEM = Standard error of mean. SMSM: sprouted moringa seed meal. MCV: mean corpuscular volume; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentration

Table 2: Haematological indices of growing rabbits fed graded levels of sprouted moringa seed meal diets.

The Serum-biochemical Profiles of Growing Rabbits fed Varying Levels of Sprouted Moringa Seed Meal Diets

The serum biochemical indices of growing rabbits fed varying levels of sprouted moringa seed meal are shown in Table 3. There was significant difference in the serum total protein obtained in the rabbits fed experimental diets with the value range from 9.29 g/dl in diet 3 to 10.39 g/dl in control diet. The serum albumin values (6.88 - 8.21 g/dl) was obtained in this study. There was significant (*P*<0.05) difference in the value of serum globulin (1.66 - 2.85 g/dl) recorded, highest value of 2.85 g/dl was recorded in diet 2 while lowest value of 1.66 g/dl was obtained in diet 3. Albumin/globulin ratio was significantly (*P*<0.05) higher in rabbits on diet 3 (4.60), the least value was observed in rabbits on diet 2 (2.41). Alanine aminotransferase (ALT), aspartate transaminase (AST) and alkaline phosphatise (ALP) were significantly (*P*<0.05) affected by varying levels of sprouted moringa seed meal. AST increased (p<0.05) linearly with increasing levels of sprouted moringa seed meal (SMSM). The AST value observed in this study ranged from 25.30 iu/l - 42.60 iu/l, highest value was observed in rabbits fed control diet while the lowest was obtained in rabbits on diet 2, these values were in agreement with the reference range of (10-98 iu/l)(MediRabbit, 2003). The values of ALT recorded were significantly (*P*<0.05) influenced by the experimental diets, the values obtained ranged between 50.70 iu/l in diet 2 and 168.00 iu/l in diet 1. The value of ALP was significantly (p<0.05) affected by the experimental diets highest value of 10.70 iu/l was recorded in control diet while lowest value of 4.00 iu/l was observed in diet 3. The cholesterol values (2.30 to 4.10 mmol/l) showed significant (*P*<0.05) differences across the treatment groups.

Parameters	Dietary Treatment				±SEM	*p*-Value
	1 (Control)	2 (SMSM 5 %)	3 (SMSM 10 %)	4 (SMSM 15 %)		
Total protein (g/dl)	10.39^{a}	9.71^{c}	9.29^{d}	10.12^{b}	1.26	0.003
Albumin (g/dl)	8.15^{a}	6.88^{c}	7.63^{b}	8.21^{a}	1.61	0.001
Globulin (g/dl)	2.24^{b}	2.85^{a}	1.66^{d}	1.91^{c}	1.35	0.002
Albumin/globulin ratio	3.64^{a}	2.41^{b}	4.60^{a}	4.30^{a}	0.28	0.023
AST (iu/l)	42.60^{a}	25.30^{d}	28.50^{c}	35.40^{b}	2.01	0.002
ALT (iu/l)	168.00^{a}	50.70^{d}	79.10^{b}	71.60^{c}	13.54	0.003
ALP(iu/l)	10.70^{a}	4.80^{b}	4.00^{b}	10.10^{a}	0.92	0.001
Cholesterol (mmol/l)	3.40^{a}	3.40^{a}	4.10^{a}	2.30^{b}	0.23	0.015

abcd Means with different superscripts on the same row were significantly (P<0.05) different. SEM = Standard error of mean. SMSM: sprouted moringa seed meal. AST: Aspartate amino transferase; ALT: Alanine amino transferase; ALP: Alkaline phosphatase

Table 3: Serum-biochemical parameters of growing rabbits fed varying levels of sprouted moringa seed meal diet.

Discussion

The PCV range of 39.00-48.00 % recorded in this study fell within the normal range of 30-50 % reported by Fudge, [13] for healthy rabbits, this is an indication that the PCV of the rabbits were not adversely affected by the experimental diets. This corroborates with the result of Ahamefule, *et al*., [14] who reported that normal PCV values are indicators of adequate nutritional status of rabbits. Melillo, [15] reported that PCV of less than 30% in rabbit

indicates anemia. In this study, there was no clinical state of anaemic condition as a result of the diets fed to the animals. The values of haemoglobin obtained were within the reference range of 9.4-17.4 g/dl according to MediRabbit [16]. Haemoglobin within the normal physiological range is an indication of improved oxygen carrying capacity of the blood which lead to a better availability of nutrients to the rabbits consequently improved the health of the animals [17]. Ahamefule *et al*., [14] reported that raw seeds contain haemagglutinin, and other ANFs which may destroy red blood cell or cause reduction in the red blood cell counts, it was also reported that reduction in red blood cell concentration below normal may cause anaemia [18]. The RBC values obtained in this study were within the normal range of (3.8 - 7.9 x 106/ul) MediRabbit [16], this implies that the ANFs in the sprouted moringa seed meal do not have detrimental effect on the health of the rabbits. The value of WBC obtained in this study were slightly below the reference range of (5.00 - 13.00 x 103/ul) as reported by MediRabbit [16] for healthy rabbits. Ewuola and Egbenike [19] observed that increase in WBC above the normal range could result from allergic conditions or presence of foreign body in circulating system. In this study, there was no case of such abnormal rise in value of WBC. This implies that the health of the rabbits was not compromised by toxin assault through the diets [20]. The value of MCV obtained in diet 2 (67.70 fl) and diet 3 (59.10 fl) were within the reference range of (50 -75 fl) as reported by Mitruka and Rawnsley [21] while there was slight increase in the value recorded for rabbit on diet 4 (80.00 fl). MCV is elevated or decreased in accordance with average red blood cell size, low MCV indicates microcytic while high MCV indicates macrocytic this is an indication that sprouted moringa seed meal has ability to maintain the red blood cell size in rabbits fed diet 2, 3 and 4 but control diet (123.80 fl) had abnormal rise in the MCV value. The MCH recorded for rabbits on diet 2 and diet 3 were within the normal range of 18-24 pg [16]. The decrease in the MCH value observed in rabbits on control diet is an indication that the animals are exposed and poorly dealing with stressors [22]. The MCHC was not significantly affected by the dietary treatments, both values from additive group and control group fell within the recommended ranges of 24.00 g/dl - 35.00 g/dl according to Simaraks, *et al*., [23]. Howlett, [24] reported that low MCHC value less than 29.0 g/dl can be attributed to iron and other trace element deficiency which indicates anaemia. This is an indication that the experimental diets fed were not detrimental to the health of the rabbits. The monocytes value observed were within the reference range of 0-4 % for healthy rabbits. The highest lymphocytes value of 84.00 % was obtained in rabbits fed control diet while lowest value of 26.00 % was recorded in diet 2, both the diet 3 and 4 had similar value of 40.00 % which is slightly below the reference range of 43-80 % [16]. This implies that the rabbits do not suffered from leukocytosis as a result of increase in the leukocyte counts outside the normal range which occur due to increase in blood cell production in attempt to combat the toxin assault in the diets [19].

Saka *et al*., [25] reported that variations in serum total protein occur due to the differences in the rate of protein metabolism and utilization by the rabbits. The values obtained were above the reference range for rabbits. According to [26, 27] it was reported that increase in serum total protein reflects the ability of the animal to store reserve protein when they have reached the maximum capacity for protein intake. The values obtained in this study were slightly increase from normal range. The serum albumin values (6.88 - 8.21 g/dl) obtained in this study was relatively similar to the range of values (4.0 to 7.2 g/dl) reported by Saleh *et al*., [28]. All the values of serum globulin obtained were within the normal range of 1.5 - 3.3 g/dl [29] for rabbit.

The value of ALP recorded for rabbits on diet 1 and 4 fell within the reference range of (10 - 96 iu/l) [29] while those on diet 2 and 3 were out of the range. It was revealed that aspartate aminotransferase (AST), alkaline phosphatase (ALP), and alanine aminotransferase (ALT) function as bio-indicators in the blood which indicate functionality of liver. Yildirim *et al*., [30]. Szabo *et al*., [31] reported that reduced level of ALP may be an indication of a slowdown of bone growth. The result of this study corroborate with the findings of Saka *et al*., [25] who reported similar cholesterol range of (2.41 – 3.48 mmol/l) in the rabbits fed moringa seed powder.

CONCLUSION

The study showed that sprouted moringa seed meal improved the health of the animals by keeping value of some of the blood components within normal physiology range for healthy rabbits. It could be concluded that inclusion of sprouted moringa seed meal up to 15 % in rabbit diets did not exert depressive effect on blood constituents.

ACKNOWLEDGEMENT

The author would like to thank the staff members of the Department of Agricultural Technology of School of Agriculture and Agricultural Technology, Federal Polytechnic, Ado-Ekiti for their support in the study.

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