

## Effect of Dietary Supplementation of Melon (*Citrullus Lanatus*) Seed Oil on the Growth Performance and Antioxidant Status of Growing Rabbits

Singh, A.S\*, Alagbe, J.O\*\*, Sharma, S\*\*\*, Oluwafemi, R.A\*\*\*\*, Agubosi, O.C.P\*\*\*\*\*

1 University of Mysore, India

2 Department of Animal Nutrition and Biochemistry, Sumitra Research Institute, Gujarat, India

3 Osmania University, India

4,5 Department of Animal Science, University of Abuja, Nigeria

P.O.Box 5205, Garki Area 10, Abuja, Nigeria (900001)

E-mail: drmusabas@gmail.com

\*\*\*

**Abstract:** This study was carried out to determine effect of dietary supplementation of melon (*Citrullus lanatus*) seed oil (WMO) on the growth performance and immune response of growing rabbits. Thirty six (36), 5-6 weeks weaner rabbit of mixed breed and sex with an average weight of 435 g – 438 grams were randomly divided into four (4) treatments of nine rabbits per group and each rabbit served as a replicate in a completely randomized design (CRD). The experiment lasted for 12 weeks and all other management practices were strictly observed. Basal diet was formulated according to the nutrient requirements of rabbit according to NRC (1977). Treatment (T1) were fed basal diet with 0 % WMO, T2, T3 and T4 were fed basal diet supplemented with WMO at 0.2 %, 0.4 % and 0.6 % respectively. Results obtained were used to examine the average daily weight gain (ADWG), average daily feed intake (ADFI), feed: gain, mortality, activities of superoxide dismutase (SOD), glutathione-S-transferase (GST), reduced glutathione (GSH) and malonyldialdehyde (MLA). ADWG, feed: gain and mortality were significantly different ( $P < 0.05$ ) among the treatments. ADFI increased as the level of WMO increases, though not at a significant level ( $P > 0.05$ ). Highest mortality was recorded among animals in T1 (1.00 %), none was recorded in the other treatments ( $P < 0.05$ ). Activities of SOD, GST, GSH and MLA

were significantly ( $P > 0.05$ ) influenced by WMO. It was concluded that dietary supplementation of WMO up to 0.6 % enhanced growth performance, improved feed: gain and had no negative effect on the antioxidant parameters of rabbits, it is safe and could be used to bridge the gap between food safety and production.

**Key words:** growth performance, feed, melon seeds, rabbits, mortality.

### Introduction

Animals encounter numerous stressors during their lives. These stressors cause hormonal changes, decrease in feed intake, altered nutrient metabolism and suppressed immune function (Gary and Richard, 2002). Animal performance is a function of genetic potential and the environment. Immune stress is loss of immune homeostasis caused by various factors including different production and environmental stressors (Sripathy, 2009). Nutrients are known to influence responses of rabbits to a disease challenge, thus immune system benefits largely from proper nutrition or feeding of animals (Alagbe, 2020; Oluwafemi et al., 2020).

Scientific reports have shown that medicinal plants and their extracts are rich in phytochemical constituents such as tannins,

alkaloids, flavonoids, terpenoids, saponins, steroids, glycosides, saponins, phenols, carbohydrates, protein and amino acids that produce significant therapeutic effects and pharmacological properties such as: antimicrobial, anti-inflammatory antiviral, antifungal, hepato-protective, miracidicidal, cytotoxic, antioxidant, immunostimulatory, neuro-protective, hypolipidemic and antispasmodic activities (Alagbe, 2017; Kondratyuk and Pezzuto, 2004; Anagnostopoulou et al., 2006; Dillard and German, 2000; Miller and Larrea, 2002; Nichenametla et al., 2006; Prakash et al., 2007). Immunomodulatory activity of plant extracts depends on various factors including nature of stressors, dosage, type of extracts and formulation used, phytochemical constituents and so on (Sripathy, 2009). World health organization (1991) reports have shown that there are over 21, 000 species of medicinal plants globally. Many of these plants and their extracts are relatively cheap, effective and safe in prolong use (Gilani, 2005). Among the potential underutilized plant are melon seeds. Melon (*Citrullus lanatus*) is an herbaceous creeping plant belonging to the family Cucurbitaceae. It can be grown in most part of the world and mainly propagated by seeds and thrives best in warm areas (Betty et al., 2016; Olaofe et al., 1994; Fokou et al., 2004; Mabalaha et al., 2007). The plant contains Citrulline which is transformed into the essential amino acid (arginine) which is vital in the synthesis of nitric oxide and strengthening of the immune and reproductive systems (Edidiong et al., 2013; Collins et al., 2007; Jacob et al., 2015). Melon seeds are rich in carbohydrates, protein, fibre, fats, minerals and other essential vitamins which can contribute substantially towards

obtaining a balanced diet (Martin, 1998; Sodeke, 2005; Omorayi and Dilworth, 2007).

The aim of this research work was to examine effect of dietary supplementation of melon (*Citrullus lanatus*) seed oil on the growth performance and immune response of growing rabbits.

### **MATERIALS AND METHODS**

#### **Experimental site**

The experiment was carried out at Division of Animal Nutrition, Sumitra Research Institute, Gujarat, India during the month of January to March, 2021.

#### **Collection and processing of test material**

Fresh, healthy and mature melons were harvested within Sumitra Research Institute, Gujarat, India. It was identified and authenticated by a certified crop taxonomist of the institute, thereafter the fruits were sliced open with a clean knife; the removed seeds were washed and sundried for 10 days. The dried samples were grinded into powder using a blender and stored into a well labeled air tight container for further analysis. Extraction of melon seed oil (WMO) was carried out according to the methods outlined by Oyeleke et al. (2012).

Crude fibre, crude protein, moisture, ether extract and moisture content were determined according with the official methods of the association of official analytical chemist (AOAC, 2000). Mineral analyses of calcium, phosphorus, potassium, sodium, magnesium, manganese, zinc, iron, cobalt, copper, chromium selenium, cadmium and lead were determined using Atomic Absorption Spectrophotometer (AAS – Model 156Y) based on (AOAC, 2000). Amino acid analysis was carried out using methods

reported by Kundan (2017).

### **Animals and their management**

Thirty six (36), 5-6 weeks weaner rabbit of mixed breed and sex with an average weight of 435 g – 438 g were purchased from a local market in India. It was randomly distributed to four treatments of nine rabbits per treatment in a completely randomized design (CRD). Animals were housed individually in a locally constructed wire cage measuring (15 × 12 × 25 cm) with provisions of clay feeding and water troughs. Rabbits were given prophylactic treatment and acclimatized for two weeks during which they fed commercial growers mash before the commencement of the experiment. Rabbits were fed twice daily at 8:00 am and 4:00 pm while clean water was given ad libitum, all other management practices were strictly observed throughout the experimental period which lasted for 12 weeks.

### **Formulation of experimental diets**

The diets contained maize, soya meal, palm kernel meal, limestone, bone meal, lysine, methionine, premix and salt. They were mixed together to formulate a basal diet according to nutritional requirement of rabbits according to NRC (1977). Treatment 1 (T1) contained basal diet + 0 % WMO, basal diet + 0.2 % WMO (T2), basal diet + 0.4 % WMO (T3) and basal diet + 0.6 % WMO (T4).

### **Measurements**

Feed intake (FI) was determined by difference between feed offered and left over.

Weight gain (g) = final weight – initial weight

Feed to gain ratio = feed intake (g)/ weight gain (g)

### **Antioxidant parameters**

Blood samples were collected from the marginal veins of the ears of three randomly selected rabbits per treatment to determine the

antioxidant status of the animal. Activities of superoxide dismutase (SOD), glutathione-S-transferase (GST), reduced glutathione (GSH) and malonyldialdehyde (MLA) were carried out using method outlined by Singh et al. (2011).

### **Statistical analysis**

All data were subjected to one -way analysis of variance (ANOVA) using SPSS (18.0) and significant means were separated using Duncan multiple range tests (Duncan, 1955). Significant was declared if  $P \leq 0.05$ .

## **RESULTS AND DISCUSSION**

### **Proximate composition of experimental diet**

Table 1 reveals the chemical composition of experimental diet. The experimental diet contained dry matter (88.01 %), crude protein (17.24 %), crude fibre (10.33 %), ether extract (3.44 %), calcium (0.88 %), phosphorus (0.41 %) and energy (2500.7 Kcal/kg). The crude protein and dry matter values obtained in this study are in agreement with the values obtained by Aduku and Olukosi (1990); Alagbe (2021) and Andrzej et al. (2019) who examined the effect of dietary supplementation of silkworm pupae meal on the performance of rabbits. Crude fiber and ether extract value is in line with the recommended range by Adham et al. (2020); Alagbe (2019). The calcium and phosphorus value obtained in this experiment were higher than the values obtained by Lima et al. (2017) but in conformity with the values obtained by Lawal et al. (2010) who determined the effect of soya meal based meal diet on the performance of Albino rats. Energy value is in close agreement with the findings of Omokore and Alagbe (2019); Onyekwere et al. (2010) when Bambara nut waste meal were fed to growing rabbits. According to Omokore and Alagbe (2019) Essential nutrients required by rabbits are those which will be able to maintain

normal physiological processes of the body such as growth, health, digestion, reproduction and lactation. Inadequate energy, protein or micronutrients in the diet may impair reproduction of rabbits (Niyi, 1997). Rabbit's nutrition and requirements for feed intake vary with age and particularly with reproductive status (Aduku and Olukosi, 1990; Alagbe and Akintayo, 2020). Proteins play a vital role in biological processes, catalyze reactions in the body, transport molecules such as oxygen, keep the body healthy as part of the immune system and transmit messages from cell to cell (Ojewuyi et al., 2014). Ether extracts or fats are very good sources of energy and aid in the transport of fat-soluble vitamins, insulate and protect internal tissues, and contribute to important cell processes (Pamela et al., 2005). Dietary fibre enhances digestion promotes digestion and reduce the risk of cardiovascular disease in animals (Musa et al., 2020). Calcium, phosphorus and other minerals are important in many biochemical reactions functioning as co-enzyme and aid physiological functioning of major metabolic processes in the body (Alagbe and Omokore, 2019). However, all the values obtained were within the nutritional requirements of rabbits according to NRC (1977).

Table 1: Percentage composition of experimental diet

Ingredients	Quantity (kg)
Maize	20.00
Wheat offal	41.00
Palm kernel meal	25.00
Soya meal	12.65
Bone meal	0.20
Limestone	0.40
Lysine	0.10
Methionine	0.10
*Growers premix	0.25

Salt	0.30
Total	100.00
<b>Calculated analysis (%)</b>	
Dry matter	88.01
Crude protein	17.24
Crude fibre	10.33
Ether extract	3.44
Calcium	0.88
Phosphorus	0.41
ME:kcal/kg	2500.7

\*Premix - quantity per kg of product: vitamin A, 2 500 000 IU; vitamin D3, 500 000 IU; biotin, 50 mg; choline, 50 mg; niacin, 10000 mg; calcium pantothenate, 3000 mg; vitamin B12, 7 mg; vitamin B2, 1800 mg; vitamin E, 7500 mg; vitamin K3, 1000 mg; Fe, 40000 mg; Cu, 35000 mg; Mn, 20000 mg; Zn, 40000 mg; Co, 360 mg; I, 840 mg; Se, 120 mg.

**Proximate composition of dried melon seed**

Proximate composition of dried water melon seed is presented in Table 2. The sample contained dry matter, moisture content, crude protein, crude fibre, ether extract, ash and energy at 91.12 %, 7.86 %, 17.40 %, 30.83 %, 25.50 %, 2.71 % and 402.7 Kcal/kg respectively. The crude protein, crude fibre and ether extract values conforms to the findings of Betty et al. (2016). The ash value was lower than those reported by Oyeleke et al. (2012) but are in close agreement with the findings of Taiwo et al. (2008). Energy value of water melon seed conforms to the findings of Alagbe (2020) who examined the proximate composition of Prosopis africana stem bark. The sample contained higher level of protein which is a clear indication that it can be used as a protein supplement in animals (NRC, 1994). The ash content gives an indication of the amount of minerals present in a particular sample, which are important in many biochemical reactions functioning as co-enzyme and aid physiological

functioning of major metabolic processes in the body (Onwuka, 2005). The energy result thus suggests that water melon seeds may not be able to supply adequate amount of calorie to animals.

Table 2: Proximate composition of dried melon seed

Constituents	Composition
Dry matter	91.12
Moisture content	7.86
Crude protein	17.40
Crude fibre	30.83
Ether extract	25.50
Ash	2.71
Energy (Kcal/100g)	402.7

Mineral composition of dried melon seed

Table 3 reveals the mineral composition of melon seed. The sample contained calcium (75.62 mg/100g), phosphorus (42.77 mg/100g), potassium (11.88 mg/100g), magnesium (26.80 mg/100g), zinc (30.81 mg/100g), sodium (19.40 mg/100g), copper (8.45 mg/100g), iron (3.61 mg/100g) and manganese (12.56 mg/100g). In order of abundance calcium > phosphorus > potassium > zinc > magnesium > sodium > manganese > copper > iron. However, all values were within the WHO (1991) recommendation. Calcium is the abundant element in the body; it is an important constituent of the skeleton and teeth, deficiency of calcium in the body results in tetany (Vasudevan and Sreekumari, 2007; Ellenberger et al., 1994). Phosphorus plays a vital role in bone formation (Alagbe, 2019). Iron is an essential trace element for haemoglobin formation and normal functional of the central nervous system and in the oxidation of carbohydrates, protein and fats (Adeyeye and Otokiti, 1999). Magnesium is major intracellular cations in cells; they were catalyst to enzymatic reactions and assimilation of

phosphorus (Vasudevan and Sreekumari, 2007; Ryan, 1991). Sodium is the major cation which is involved in maintaining osmotic pressure, controlling water balance and acid-base balance (Akpanyung, 2005). It also functions in muscle contractions, nerve impulse transmission and glucose / amino acid transport (Oduye and Fasanmi, 1971). Zinc serves as a cofactor in many enzyme systems, including arginase, enolase, several peptidases, and oxalacetic decarboxylase (Alagbe, 2016). Manganese is a cofactor or component of several key enzyme systems, manganese is essential for bone formation (re. mucopolysaccharide synthesis), the regeneration of red blood cells, carbohydrate metabolism, and the reproductive cycle (Okwu, 2005). Copper is involved with iron metabolism, and therefore haemoglobin synthesis and red blood cell production and maintenance (Ishida et al., 2000).

Table 3: Mineral composition of dried melon seeds

Parameters	Composition (mg/100g)	WHO range (mg/100g)
Calcium	75.62	36.00 – 80.00
Phosphorus	42.77	20.00 – 45.00
Potassium	11.88	10.00 - 25.00
Magnesium	26.80	-
Zinc	30.81	15.00 – 50.00
Sodium	19.40	4.00 – 50.00
Copper	8.45	10.00 – 30.00
Iron	3.61	-
Manganese	12.56	10.00 – 20.00

Amino acid of dried melon seeds

The amino composition of melon seeds is presented in Table 4. The sample contains aspartic acid, glutamic acid, arginine, serine, alanine, phenylalanine, glycine, threonine, tyrosine, valine, proline, methionine, lysine, isoleucine, leucine and histidine at 2.11g/100g, 1.88 g/100g, 3.85 g/100g, 0.67 g/100g, 1.33 g/100g, 0.62 g/100g, 1.21 g/100g, 0.72 g/100g,

0.41 g/100g, 0.26 g/100g, 0.54 g/100g, 0.31 g/100g, 0.22 g/100g, 0.68 g/100g, 0.74 g/100g and 0.69 g/100g respectively. The values obtained in this study are in agreement with the values obtained by Edgar et al. (2014); Kasimu et al. (2015) who examined the lipid and proximate composition in Anisophyllea boehmii seeds. According to Perez and Avalos (2009); Cuin and Shabala (2007), amino acids play an important role in the synthesis of protein and precursors in the formation of secondary metabolism molecules that participate in cell signaling, homeostatis and gene expression. It also participates in various physiological processes such as skeletal muscle function, atrophic conditions, sarcopenia and cancer (Wu, 2009; Nicastro et al., 2011).

Table 4: Amino acid profile of melon seeds

Constituents	Composition (g/100g)
Aspartic acid	2.11
Glutamic acid	1.88
Arginine	3.85
Serine	0.67
Alanine	1.33
Phenylalanine	0.62
Glycine	1.21
Threonine	0.72
Tyrosine	0.41
Valine	0.26
Proline	0.54
Methionine	0.31
Lysine	0.22
Isoleucine	0.68
Leucine	0.74
Histidine	0.69

**Performance characteristics of growing rabbits fed diets supplemented with WMO**

Performance characteristics of growing rabbits fed diets supplemented with WMO is presented in Table 5. Initial body weight (IBW), final body

weight (FBW), weight gain (WG), average daily weight gain (ADWG) and feed: gain ranged between 435.0 – 438.0 g, 986.1 – 1170.6 g, 548.4 – 735.6 g, 9.14 – 12.51 g and 7.18 – 7.62 respectively. Total feed intake (TFI) and average daily feed intake (ADFI) ranged between 7200.1 – 7308.4 g and 120.7 – 123.0 g. WG and feed: gain were significantly (P<0.05) different among the treatments. The result obtained is in agreement with the findings of Olatunji et al. (2016); Alagbe et al., 2020; Oluwafemi et al. (2020); Alagbe and Oluwafemi (2019) who evaluated the growth performance of weaner rabbits fed Noni (Morinda citrifolia) and Moringa olifera leaf mixture as partial replacement of soya bean meal. The highest weight gain observed in T3 and T4 could be attributed to the presence of phytochemicals in WMO. According to Olafadehan et al. (2020); Kim et al. (2015), phytochemicals are performs multiple biological activities such as antimicrobial, antifungal, antiviral, anti-inflammatory and antioxidant properties. In addition, the inclusion of phytochemicals in the diets alters and stabilizes the intestinal microbiota and reduces microbial toxic metabolites in the gut, owing to their direct antimicrobial properties on various pathogenic bacteria, which results in relief from intestinal challenge and immune stress, thus improving performance. Average daily feed intake increased from diet 1 to 4 though not at a significant level (P>0.05). This is a clear indication that WMO is capable of improving the palatability of feed (Akintayo and Alagbe, 2020). Highest mortality was recorded in T1 and none was recorded in the other treatments (P<0.05).

Table 5: Performance characteristics of growing rabbits fed diets supplemented with WMO

Parameters	T1	T2	T3	T4
------------	----	----	----	----

					SEM
IBW (g)		438.0			
	437.7		435.8	435.0	5.11
FBW (g)	986.1 <sup>b</sup>	1012.8 <sup>b</sup>	1168.0 <sup>a</sup>	1170.6 <sup>a</sup>	9.33
WG (g)	548.4 <sup>b</sup>	574.8 <sup>b</sup>	732.2 <sup>a</sup>	735.6 <sup>a</sup>	2.71
ADWG (g)	9.14 <sup>b</sup>	9.60 <sup>b</sup>	12.20 <sup>a</sup>	12.51 <sup>a</sup>	0.04
TFI (g)	7200.1	7306.2	7308.0	7308.4	10.90
ADFI (g)	120.7	121.8	122.9	123.0	2.30
Feed: gain	7.62 <sup>a</sup>	7.55 <sup>a</sup>	7.20 <sup>b</sup>	7.18 <sup>b</sup>	1.22
Mortality	1.00	-	-	-	

Means in the same row with different superscripts differ significantly (P<0.05)

Initial body weight (IBW), final body weight (FBW), weight gain (WG), average daily weight gain (ADWG)

### Antioxidant response of growing rabbits fed diets supplemented with WMO

Table 6 reveals the antioxidant response of growing rabbits fed diet supplemented with WMO. Malanodialdehyde (MDA), superoxide dismutase (SOD), glutathione-S-transferase (GST) and reduced glutathione (GSH) ranged between 1.22 – 3.87 (U/mg Hb), 28.1 – 42.1 (U/mg Hb), 12.1- 30.8 (U/mg Hb) and 29.7 – 40.6 (U/mg Hb) respectively. The parameters follow similar pattern and values were highest in T3, T4, intermediate in T2 and lowest in T1 (P<0.05). This result is in conformity with the findings of Mahipal et al. (2015). According to Hasanuzzaman et al. (2015); Jackson et al. (1978), plants have lot of antioxidant systems that are capable of scavenging free radicals. SOD is a ubiquitous metalloenzymes that constitute the first line of defense against reactive oxygen species, it constitutes one of the major enzymatic components of detoxification of superoxide radicals generated in biological

system by catalyzing its dismutation to H<sub>2</sub>O<sub>2</sub> and finally H<sub>2</sub>O and O<sub>2</sub> by catalase and peroxidase (Mukesh and Chet, 2000; Hernandez et al., 2004; Gill and Tuteja, 2010; Fridovich, 1975). Adequate availability of glutathione is critical in maintaining health, protecting the body from toxins and promoting longevity in animals (Pompella et al., 2009; Kern et al., 2011; Arosio et al., 2002). Malondialdehyde is usually used as a biomarker for many health problems such as respiratory and cardiovascular diseases (Maryam et al., 2015). Glutathione directly scavenges diverse: superoxide anion, nitric oxide, hydroxyl and carbon radicals, protects cells from oxidants via recycling vitamin C and catalytically detoxifies: hydroperoxides, perxynitrites and lipid peroxides (Julius et al., 1994; Barja et al., 2000; Allen et al., 2011).

Table 6: Antioxidant response of growing rabbits fed diets supplemented with WMO

Parameters	T1	T2	T3	T4	SEM
MDA (U/mg Hb)	1.22 <sup>c</sup>	2.91 <sup>b</sup>	3.03 <sup>b</sup>	3.87 <sup>a</sup>	0.21
SOD (U/mg Hb)	28.1 <sup>c</sup>	32.6 <sup>b</sup>	41.0 <sup>a</sup>	42.1 <sup>a</sup>	0.03
GST (U/mg Hb)	12.1 <sup>b</sup>	19.5 <sup>b</sup>	26.1 <sup>a</sup>	30.8 <sup>a</sup>	1.21
GSH (U/mg Hb)	29.7 <sup>c</sup>	30.8 <sup>b</sup>	38.0 <sup>a</sup>	40.6 <sup>a</sup>	0.97

Means in the same row with different superscripts differ significantly (P<0.05)

GST: glutathione-S-transferase; SOD: superoxide dismutase; MDA: malondialdehyde; GSH: reduced glutathione.

### Conclusion

Medicinal plants are rich in secondary metabolites which are potential sources of

drugs and essential oils of therapeutic importance. Essential oils are cheap, safe, effective and easily available. Dietary supplementation of WMO in rabbits is capable of performing several pharmacological activities which includes: antioxidant, antimicrobial, anti-inflammatory, hepato-protective, hypolipidemic, cytotoxic etc. Supplementation of WMO at 0.6 % in rabbit diets is capable of improving growth performance without any deleterious effect on the immune system of the animal.

## References

1. Lima P.J.D.O., Watanabe P.H., Cândido R.C., Ferreira A.C.S., Veira A.V., Rodrigues B.B.V., Nascimento G.A.J., Freitas E.R. (2017). Dried brewers grains in growing rabbits: nutritional value and effects on performance. *World Rabbit Sci.* 2017, 25: 251-260.
2. Adham A. AL-Sagheer, Gamal Abdel-Rahman, Mohamed S. Ayyat, Hassan A. Gabr & Gihan F. Elsis. (2020). Productive performance response of growing rabbits to dietary protein reduction and supplementation of pyridoxine, protease, and zinc. *An Acad Bras Cienc* (2020) 92(3): e20180989 DOI 10.1590/0001-3765202020180989.
3. Andrzej, G., Janusz, S and Dorota, K. (2019). Growth performance and meat composition of rabbits fed diets supplemented with silkworm pupae meal. *Spanish Journal of Agricultural Science.* 17(3) e0607.
4. Alagbe, J.O and Akintayo-Balogun, O.M. (2020). Effects of dietary supplementation of Albizia lebeck seed oil (ALO) on the fatty acid composition of weaner rabbits. *Biochemistry and Biotechnology Research*, 8(2): 29-33.
5. Musa, B., Alagbe, J.O., Adegbite Motunrade Betty, Omokore, E.A. (2020). Growth performance, caeca microbial population and immune response of broiler chicks fed aqueous extract of *Balanites aegyptiaca* and *Alchornea cordifolia* stem bark mixture. *United Journal for Research and Technology*, 2(2):13-21.
6. Omokore, E.O and Alagbe, J.O. (2019). Efficacy of dried *Phyllanthus amarus* leaf meal as an herbal feed additive on the growth performance, haematology and serum biochemistry of growing rabbits. *International Journal of Academic Research and Development.* 4(3): 97-104.
7. Alagbe, J.O. (2019). Performance and haemato-biochemical parameters of weaner rabbits fed diets supplemented with dried water melon (rind) meal. *Journal of Dairy and Veterinary Sciences.* 8(4):001-007.
8. Alagbe, J.O and Omokore, E.A. (2019). Effect of replacing soya bean meal with *Indigofera zollingeriana* leaf meal on the performance and carcass characteristics of growing rabbits. *International Journal of Multidisciplinary Research and Development.* 6(5): 74-77.
9. Pamela, C. C., Richard, A. H. and Denise, R. F. (2005). *Lippincotts illustrated Reviews Biochemistry* 3rd ed., Lippincott Williams and Wilkins, Philadelphia. 335- 388.
10. Ojewuyi, O.B, Ajiboye, T. O, Adebajo, E. O, Balogun, A, Mohammed, A.O. (2014). Proximate composition, phytochemical and mineral contents of young and mature *Polyalthia longifolia* Sonn.leaves. *Fountain Journal of Natural and Applied Sciences:* 2014; 3(1): 10 – 19.
11. Niyi, A. (1997). Prospects of Commercial Rabbit Keeping in Nigeria *Livestock Echo* April- June Pp. 51-54.
12. NRC, (1977) *Nutrient Requirement of Domestic Animal, Nutrient Requirement of Rabbits.* Second Edition National Academy of Science Washington D.C.



13. Onwuka, G. I. (2005). Food Analysis and Instrumentation; Theory and Practice Naphthalic prints, Surulere, Lagos, Nigeria. 219- 230.
14. Alagbe, J.O (2020). Chemical evaluation of proximate, vitamin and amino acid profile of leaf, stem bark and roots of *Indigofera tinctoria*. International Journal on Integrated Education. 3(10): 150-157.
15. Alagbe, J.O (2020). Proximate, phytochemical and vitamin compositions of *Prosopis africana* stem bark. European Journal of Agricultural and Rural Education. 1(4): 1-7.
16. Duncan, D.B. (1955). Multiple range and multiple F-test. *Biometrics* 11(1):1-42.
17. National Research Council (1994). Nutrient requirement of poultry 9th Rev Edn, Washington D.C. National Academy Press.
18. A.O.A.C (2000). Association of Official Analytical Chemists. Official Methods of Analysis 19th Edition Washington, D.C Pages 69-77.
19. Alagbe, J.O., Shittu, M.D., Bamigboye, S.O and Oluwatobi, O.A. (2019). Proximate and mineral composition of *Pentadiplandra brazzeana* stem bark. *Electronic Research Journal of Engineering, Computer and Applied Sciences*. 1(2019):91-99.
20. Vasudevan, M. D. and Sreekumari, S. (2007). Textbook of biochemistry for medical students. 5th Ed., Jaypee Brothers Medical Publishers (P) Ltd. New Delhi, India, pp. 283-287, 309-313, 318-320, 322.
21. Akpanyung, E. O. (2005). Proximate and mineral composition of bouillon cubes produced in Nigeria. *Pak. J. Nut.* 4 (5), 327-329.
22. Adeyeye, E. and Otokili, M. K. (1999). Proximate composition and some nutritionally valuable minerals of two varieties of *Capsicum annum* (Bell and Cherry Peppers). *Discov. Innov.* 11: 75-81.
23. Ryan, M. F. (1991). The role of magnesium in clinical biochemistry: an overview. *Ann. Clin. Biochem.* 28:19-26.
24. Olaleye, M. T. (1997). The mineral elements proximate analysis, phytochemical screening and toxicants of *vernonia amygelina* (Etidot) and *Marsdenia latifolia* (Utasi)- unpublished, B. Sc. Project Biochemistry department, University of Uyo, Uyo.
25. Alagbe, J.O. (2016). Effect of heavy metals contamination on performance, blood profile of broiler chicks fed Corn-soya diet. *International Journal of Advanced Biological Research*. 6(4): 538-542.
26. Okwu, D. E. (2005). Phytochemical, Vitamins and Mineral Composition of two Nigeria Medicinal plants. *International Journal of Molecular medicine and Advance sciences*, 1(4):375- 381.
27. Ishida, H., Suzuno, H, Sugiyama, N., Innami, S. Todokoro, T. and Maekawe, A. (2000). Nutritional evaluation of chemical component of leaves stalks an stem of sweet potatoes (*Ipomea batata* Poir). *Food Chemistry*, 68: 359-367.
28. Olatunji, A.K., Alagbe, J.O and Hamed, M.A. (2016). Effects of varying levels of *Moringa olifera* leaf meal on performance and blood profile of weaner rabbits. *International Journal of Science and Research*. 5(6):803-806.
29. Oluwafemi, R.A., Egwuiyi. G.N and Alagbe, J.O. (2020). Effect of feeding *Polylathia longifolia* leaf meal as partial replacement of wheat offal. *European Journal of Agricultural and Rural Education*. 1(1), 8-16.
30. Alagbe, J.O and Oluwafemi, R.A. (2019). Growth performance of weaner rabbits fed Noni (*Morinda citrifolia*) and *Moringa olifera* leaf mixture as partial replacement of soya bean meal. *International Journal of Advanced Biological and Biomedical Research*. 7(2): 185-195.
31. Olafadehan, O.A., Oluwafemi, R.A and Alagbe, J.O. (2020). Carcass quality, nutrient retention and caeca microbial population of broiler chicks administered Rolfe (*Daniellia*

- oliveri) leaf extract as an antibiotic alternative. *Journal of Drug Discovery*. 14(33):146-154.
32. Olafadehan, O.A., Oluwafemi, R.A and Alagbe, J.O. (2020). Performance, haemato-biochemical parameters of broiler chicks administered Rolfe (*Daniellia oliveri*) leaf extract as an antibiotic alternative. *Advances in Research and Reviews*, 2020, 1:4.
33. Mahipal, C., Ashok, K.P., Shalini, B., Narayan, D., Sunil, E.J and Kusumakar, S. (2015). Dietary supplementation of a novel pathogenic feed additive: effect on nutrient metabolism, antioxidant status and immune response of goats. *Animal Production Science*. 46: 540-550 <http://dx.doi.org/10.1071/AN14770>.
34. Singh, V.K., Pattanaik, A.K., Sharma, K and Saini, M. (2011). Effects of dietary energy intake on erythrocytic, antioxidant defense in growing lambs fed wheat straw based diets. *Animal Production Science*. 51:642-649.
35. Alagbe, J.O., Agubosi, O.C.P., Ajagbe, A.D, Shittu, M.D and Akintayo Balogun, O.M (2020). Performance, haematology and serum biochemical parameters of growing grass cutters fed *Phyllanthus amarus* and *Piliostigma thonningii* leaf meal mixture as partial replacement for Soya bean meal. *United International Journal for Research and Technology*, 2(1): 14-23.
36. World Health Organization (1991). Guidelines for elemental concentrations. *Journal of American Medicine*, 23(3): 299-305,
37. Maryam, K., Khalil, A and Abolghasem, J. (2015). Reliability of Malondialdehyde as a biomarker of oxidative stress in psychological disorders. *Bioimpacts* 5(3): 123-127.
38. Edgar, F.M., Orlando, T.O., Geida, A.Y., Zamora, A., Norma, S.C., Jose, G.S.O and Jesus, R.R. (2014). Determination of Amino acids in medicinal plants from South Sonora, Mexico. *Tropical Journal of Pharmaceutical Research*. 13(4):601-606.
39. Jacob, A.G., Etong, D. I and Tijjani, A. (2015). Proximate, mineral and anti-nutritional compositions of melon (*Citrullus lanatus*) seeds. *British Journal of Research*. 2(5): 142-151.
40. Betty, T., Jacob, K.A., Faustina, D and Elsa, I.O. (2016). Water melon seeds as food: nutrient composition, phytochemicals and antioxidant activity. *International Journal of Nutrition and Food Sciences*. 5(2): 139-144.
41. Oyeleke, G.O., Olagunju, E.O and Ojo, A. (2012). Functional and physicochemical properties of water melon seed and seed oil. *IOSR Journal of Applied Chemistry*, 2(2): 29-31.
42. Ojieh, G.C., Oluba, O.M., Ogunlowo, Y.R and Orole, R.T. (2006). Comparative study on the chemical and functional properties of *Citrullus linatus* seed. *The internet Journal of Nutrition and Wellness*. 6(1): 1-5.
43. Taiwo, A.A., Agbotoba, M.O., Oyedepo, A., Shobo, O.A., Olawuni, M.O. (2008). Effects of drying methods on properties of water melon seed oil. *AJFAND* 8(4):492-501.
44. Alagbe, J.O., Sharma, R., Eunice Abidemi Ojo, Shittu, M.D and Bello Kamoru Atanda (2020). Chemical evaluation of the proximate, minerals, vitamins and phytochemical analysis of *Daniellia oliveri* stem bark. *International Journal of Biological, Physical and Chemical Studies*, 2(1):16-22.
45. Oluwafemi, R.A., Isiaka Olawale and Alagbe, J.O. (2020). Recent trends in the utilization of medicinal plants as growth promoters in poultry nutrition- A review. *Research in: Agricultural and Veterinary Sciences*. 4(1): 5-11.
46. Alagbe, J.O. (2017). Effect of dietary inclusion of *Polyalthia longifolia* leaf meal as phytobiotic compared with antibiotics on the nutrient retention, immune response and serum biochemistry of broiler chicken.

- Greener Journal of Agricultural Sciences. 7(3):74-81.
47. Alagbe, J.O. (2017). Performance, blood profile and carcass evaluation of growing grass cutters fed diets supplemented with matured *Polyalthia longifolia* leaf meal. *Scholarly Journal of Agricultural Science*. 7(2):44-49.
48. Gilani, A.R. (2005). Trends in ethnopharmacology. *Journal of Ethnopharmacology*. 100: 43-49.
49. Miller, N.J and Larrea, M.B.R. (2002). Flavonoids and other plant phenols in the diets: their significance as antioxidants. *Journal of Nutr. Environ. Med*. 12: 39-51.
50. Prakash, D and Gupta, K.R. (2009). The antioxidant phytochemicals of nutraceutical importance. *The open Nutraceuticals Journal*. 2:20-35.
51. Dillard, C.J and German, J.B. (2000). Review phytochemicals: nutraceuticals and human health. *Journal of Food Agric*. 80:1744-1756.
52. Anagnostopoulou, M.A., Kefalas, P., Papageorgiou, A.N., Assimopoulou, D. (2006). Radical scavenging activity of various extracts and fractions of sweet orange peel. *Food Chem*. 94:19-25.
53. Prakash, D., Dhakarey, R and Mishra, A. (2004). Carotenoids: the phytochemicals of nutraceutical importance. *Indian. J. Agric. Biochem*. 17: 1-8.
54. Teixeira FK, Menezes-Benavente L, Galvão VC, Margis-Pinheiro M. *Multigene* (2005). families encode the major enzymes of antioxidant metabolism in *Eucalyptus grandis* L. *Genet Mol Biol*. 28(3):529-538.
55. Marí M, Morales A, Colell A, García-Ruiz C, Fernández-Checa JC. (2009). Mitochondrial glutathione, a key survival antioxidant. *Antioxid Redox Signal*. 11(11):2685-2700. doi: 10.1089/ARS.2009.2695.
56. Filomeni G, Aquilano K, Civitareale, et al. (2005). Activation of c-Jun-N-terminal kinase is required for apoptosis triggered by glutathione disulfide in neuroblastoma cells. *Free Radic Biol Med*. 39(3):345-354.
57. Marí Marí M, Morales A, Colell A, García-Ruiz C, Fernández-Checa JC. (2009). Mitochondrial glutathione, a key survival antioxidant. *Antioxid Redox Signal*. 11(11):2685-2700. doi: 10.1089/ARS.2009.2695.
58. Julius M, Lang CA, Gleiberman L, Harburg E, DiFranceisco W, Schork A. (1994). Glutathione and morbidity in a community-based sample of elderly. *J Clin Epidemiol*. 47(9):1021-1026.
59. Barja G, Herrero A. Oxidative damage to mitochondrial DNA is inversely related to maximum life span in the heart and brain of mammals. *FASEB J*. 2000;14(2):312-318
60. Pompella A, Emdin M, Franzini M, Paolicchi A. (2009). Serum gamma-glutamyltransferase: linking together environmental pollution, redox equilibria and progression of atherosclerosis? *Clin Chem Lab Med*. 47(12):1583-1584.
61. Allen J, Bradley RD. (2011). Effects of oral glutathione supplementation on systemic oxidative stress biomarkers in human volunteers. *J Altern Complement Med*. 17(9):827-833.
62. Kern JK, Geier DA, Adams JB, Garver CR, Audhya T, Geier MR. A (2011). clinical trial of glutathione supplementation in autism spectrum disorders. *Med Sci Monit*. 17(12):CR677-CR682.
63. Arosio E, De Marchi S, Zannoni M, Prior M, Lechi A. (2002). Effect of glutathione infusion on leg arterial circulation, cutaneous microcirculation, and pain-free walking distance in patients with peripheral obstructive arterial disease: a randomized, double-blind, placebo-controlled trial. *Mayo Clin Proc*. 77(8):754-759.
64. Hernandez JA, Escobar C, Creissen G, Mullineaux P. (2004). Role of hydrogen peroxide and the redox state of ascorbate in the induction of antioxidant enzymes in pea leaves under excess light stress. *Functional Plant Biology*. 31:359-368

65. Gill SS, Tuteja N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiology and Biochemistry*. 48:909-930.
66. Hasanuzzaman M, Hossain MA, daSilva JAT, Fujita M. (2012). Plant responses and tolerance to abiotic oxidative stress: Antioxidant defense is a key factor. In: Bandi V, Shanker AK, Shanker C, Mandapaka M, editors. *Crop Stress and its Management: Perspectives and Strategies*. Berlin: Springer; 2012. pp. 261-316
67. Fridovich I. (1975). Superoxide dismutase. *Annual Review of Biochemistry*. 44:147-159.
68. Jackson CA, Moore AL, Halliwell B, Foyer CH, Hall DO. (1978). Subcellular localization and identification of superoxide dismutase in the leaves of higher plants. *European Journal of Biochemistry*. 91:339-344.
69. Lawal, W.B, Makinde, O.T., Obiageli, V., Nnajiolor, B., Ngozi, B and Abu, O.A. (2010). Organ weight, haematology and histopathology of albino rats fed soya bean meal with or without supplementing with mannanase. *Proceedings 35th Conference Nigeria Society of Animal Production, 14th – 17th March, 2010. University of Ibadan, 98-100.*
70. Onyekwere, M.U., Olabode, A.D., Okechukwu, S.O and Iheukwere, F.C. (2010). Effect of feeding boiled bambara nut waste on performance, haematology and serum biochemistry of weaned rabbits. *Proceedings 33rd Conference of Nigerian Society of Animal Production, 14-17th March, 2010, University of Ibadan. Pp. 203.*
71. Aduku, O.A and Olukosi, J.O. (1990). *Rabbit production in the tropics*. Abuja, Nigeria. Global Union Publications.