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

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ABSTRACT

This study was carried out to determine the effect of dietary supplementation of melon (*Citrallus 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. The basal diet was formulated according to the nutrient requirements of the rabbit according to NRC (1977). Treatment (T1) was 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 malonyl dialdehyde (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 ($P > 0.05$). The 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.

INTRODUCTION

Animals encounter numerous stressors during their lives. These stressors cause hormonal changes, a 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 the loss of immune homeostasis caused by various factors including different production and environmental stressors (Sripathy, 2009). Nutrients are known to influence the responses of rabbits to a disease challenge, thus the 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). The 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 parts 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). This research work aimed to examine the effect of dietary supplementation of melon (*Citrullus lanatus*) seed oil on the growth performance and immune response of growing rabbits.

METHODS

Experimental site

The experiment was carried out at the Division of Animal Nutrition, Sumitra Research Institute, Gujarat, India from 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 in a well-labeled

airtight 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 to 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 to formulate a basal diet according to the 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 the difference between feed offered and leftover.

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 malonyl dialdehyde (MLA) were carried out using the 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 the 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 are 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. The energy value is in close agreement with the findings of Omokore and Alagbe (2019); Onyekwere *et al.* (2010) when Bambara nut waste meals 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 the 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 reduces 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
Calculated analysis (%)	
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

The proximate composition of dried watermelon 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 conform to the findings of Betty et al. (2016). The ash value was lower than those reported by Oyeleke et al. (2012) but is in close agreement with the findings of Taiwo et al. (2008). The energy value of watermelon seed conforms to the findings of Alagbe (2020) who examined the proximate composition of *Prosopis africana* stem bark. The sample contained a 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 indicates the number 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 watermelon seeds may not be able to supply an adequate amount of calories 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 the 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 an 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 hemoglobin formation and normal function of the central nervous system and in the oxidation of carbohydrates, protein, and fats (Adeyeye and Otokiti, 1999). Magnesium is a major intracellular cation in cells; they were the catalyst to enzymatic reactions and assimilation of phosphorus (Vasudevan and Sreekumari, 2007; Ryan, 1991). Sodium is the major cation that 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 hemoglobin 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

The 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, homeostasis, 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 are presented in

Table 5. Performance Characteristics of Growing Rabbits Fed Diets Supplemented with WMO

Parameters	T1	T2	T3	T4	SEM
IBW (g)	437.7	438.0	435.8	435.0	5.11
FBW (g)	986.1 ^b	1012.8 ^b	1168.0 ^a	1170.6 ^a	9.33
WG (g)	548.4 ^b	574.8 ^b	732.2 ^a	735.6 ^a	2.71
ADWG (g)	9.14 ^b	9.60 ^b	12.20 ^a	12.51 ^a	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 ^a	7.55 ^a	7.20 ^b	7.18 ^b	1.22
Mortality	1.00	-	-	-	

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

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 was 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 perform 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 an 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). The highest mortality was recorded in T1 and none was recorded in the other treatments ($P < 0.05$).

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 a diet supplemented with WMO. Malondialdehyde (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 a similar pattern and values were highest in T3, T4, intermediate in T2, and lowest in T1 ($P < 0.05$). This result conforms with the findings of Mahipal *et al.* (2015). According to Hasanuzzaman *et al.* (2015); Jackson *et al.* (1978), plants have a lot of antioxidant systems that are capable of scavenging free radicals. SOD is 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 the biological system by catalyzing its dismutation to H_2O_2 and finally H_2O and O_2 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, peroxynitrites, 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 ^c	2.91 ^b	3.03 ^b	3.87 ^a	0.21
SOD (U/mg Hb)	28.1 ^c	32.6 ^b	41.0 ^a	42.1 ^a	0.03
GST (U/mg Hb)	12.1 ^b	19.5 ^b	26.1 ^a	30.8 ^a	1.21
GSH (U/mg Hb)	29.7 ^c	30.8 ^b	38.0 ^a	40.6 ^a	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 include: 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.

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