

Effect of dietary supplementation of *Albizia lebbbeck* seed oil on the fatty acid composition of weaned rabbits

Alagbe J. O.^{1*} and Akintayo-Balogun O. M.²

¹Sumitra Research Institute, Gujarat, India.

²University of Abuja, Nigeria.

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ABSTRACT

An experiment was carried out to determine the effects of dietary supplementation of *Albizia lebbbeck* seed oil (ALO) on the fatty acid composition of weaner rabbits. Fifty (50) weaned rabbits of mixed breed and sexes, aged between 6-7 weeks with an average initial body weight of 460 ± 1.3 g were randomly assigned into five dietary treatments of ten rabbits per group; each group was further divided into 5 replicates consisting of two rabbits each. Basal diet was formulated to meet the nutritional requirements of rabbits according to NRC (1977). Rabbits in treatment 1, 2, 3, 4 and 5 were supplemented with ALO at 0, 0.1, 0.2, 0.3 and 0.4% respectively. Feed and water were given *ad libitum* and the experiment lasted for 12 weeks. The results showed that significant differences ($P < 0.05$) were observed in saturated fatty acid (SFA), polyunsaturated fatty acid (PUFA) and omega-6/omega-3 ratio (n-6:n-3) values obtained. Rabbits fed diet containing 0.4% ALO had the highest PUFA value (54.17%), followed by Treatment 4 (T4) (53.01%), T3 (45.13%), T2 (37.61%) and T1 (26.93%) respectively. Similarly (n-6:n-3) composition in T5 (3.65 %) increased in T5 fed 0.4% ALO compared with T1 (1.38%) fed 0% ALO. Antherogenic index were significantly ($P < 0.05$) different among the treatments. It can be concluded that supplementation of ALO at 0.4% highly influenced the composition of fatty acid in rabbit meat.

Keywords: Rabbit, *Albizia lebbbeck* seed oil, essential oil, fatty acid.

*Corresponding author. E-mail: demsonfarms@yahoo.com.

INTRODUCTION

The use of essential oil as feed additives in livestock production is becoming an interest since the ban on the use of antibiotics by the European Union in 2006, since it alleviates the problem of antimicrobial resistance, residues in animal products and the danger posed to human health.

Essential oils (EOs) are mixture of fragrant, safe and volatile compounds, which are usually originated from plants and are named with the aromatic characteristics considering the origin of plants (Oyen and Dung, 1999). It has also been reported to contain several bioactive or phytochemicals which confer it the ability to perform multiple biological activities such as: antimicrobial,

antiviral, antifungal, antiparasitic and antidiarrheal because of the presence of tannins, flavonoids, saponins, phenols and alkaloids (Enzo, 2007; Mohammed et al., 2012). Phytochemicals which are also known phytochemicals are natural bioactive compounds that are derived from plants and incorporated into animal feed to enhance productivity (Hyun et al., 2018). The antimicrobial and modulatory properties of EOs are recent focus for agricultural applications because of a desire on the part of livestock farmers to reduce the use of unnatural chemicals in feed production (William et al., 2004).

EOs consists of two compounds, the terpenes and

phenylpropenes; their concentration varies according to the plant, parts of the plant, geographical origin, season and processing techniques (Lee et al., 2004; Hyun et al., 2018). They also play a role in appetite stimulation, improvement of enzyme secretion related to food digestion and immune activation (Gopal and Asmita, 2014) especially oil obtained from *Albizia lebbbeck*.

Albizia lebbbeck is a large deciduous tree belonging to the family Leguminosae (Fabaceae). The plant is found in West Africa and some parts of Asia including India. According to Everist (1986) *A. lebbbeck* tree ranges from medium to large of multi-stemmed spreading habit. The leaves are traditionally used for feeding ruminant animals and are also used for the treatment of asthma, malaria, conjunctivitis and diarrhea (Mohammed et al., 2012) and its seeds are easily extracted from the pods by hand or by crushing the pods and winnowing to obtain the oil (Mohammed et al., 2012) and also good sources of carbohydrates, proteins, fat soluble vitamins minerals and fatty acids. The seed oil is abundant in oleic and linoleic acid at 5.3 and 78.5% respectively (Waheed et al., 2000). *A. lebbbeck* seed contains alkaloids, saponins, tannins and flavonoids which have a high therapeutic value (Kirsti et al., 2010).

Previous research has proven that changes in the lipid composition of animal diet can alter the composition of meat fat (Bourre, 2004). Wood et al. (1999) revealed that modification of fatty acid via feeding provided the best results in monogastric animals. So many essential oils have been experimented in animals, for instance, *Centella asiatica* oil (Oyedeji and Afolayan, 2005), linseed oil (Trebusak et al., 2014), flaxseed oil (Kamran et al., 2018), garlic oil (Busquet et al., 2005), thymol and cinnamaldehyde oil (Cerisuelo et al., 2014) and most recently *A. lebbbeck* oil. The use of *A. lebbbeck* essential oil will bridge a gap between food safety and livestock production.

Therefore this experiment was designed to determine the effect of dietary supplementation of *A. lebbbeck* seed oil on the fatty acid composition of weaner rabbits.

MATERIALS AND METHODS

Site of the experiment

The experiment was carried out at Division of Animal Nutrition, Sumitra Research Farm, Gujarat, India during the month of December to February, 2020.

Collection of test material and preparation

Healthy seeds of *A. lebbbeck* were harvested within the farm premises in India; they were later authenticated at the Herbarium unit of Department of Biological Sciences on the farm and assigned a voucher specimen number MM-1256 AL. The seeds were separated manually from their coats and sundried for one (1) week. The dried seeds were granulated into coarse particles using a grinder in the laboratory. *A. lebbbeck* oil (ALO) was extracted from the coarse particles using soxhlet extraction method. It was later

poured into air tight labeled container and subjected to further analysis.

Experimental animals and their management

Fifty (50) weaned rabbits of mixed breed and sexes, aged between 6-7 weeks with an average initial body weight of 460 ± 1.3 g were obtained from a commercial farm in India and used in the experiment. Before the commencement of the experiments, animals were given prophylactic treatment of Ivermectin injection at the rate of 0.2 ml/rabbit administered subcutaneously and broad spectrum antibiotics (Oxytrox L. A[®]), multivitamins (Biovit super[®]) were given intramuscularly at the rate of 0.2 ml and 0.1 ml/rabbit respectively. The rabbits were housed individually in a special all wired cages of dimension 60 cm x 60 cm (length and width) equipped with concentrate drinkers and feeders. Five dietary treatments of ten rabbits per group were used for this experiment. The animals were allowed one-week adjustment period during which they were fed with basal diet. Feed and water were given *ad libitum* by 7:00 am and 17:00 pm and all management practices were strictly observed throughout the experimental period which will last for 12 weeks.

Experimental set-up

Experimental diet was formulated to meet the nutritional requirements of rabbits according to NRC (1977) as presented in Table 1.

T1: Basal diet + 0.0 % ALO

T2: Basal diet + 0.1 % ALO

T3: Basal diet + 0.2 % ALO

T4: Basal diet + 0.3 % ALO

T5: Basal diet + 0.4 % ALO

Measurements

Feed intake was recorded daily and body weight gain was recorded weekly, feed conversion ratio was calculated by dividing the total feed intake by weight gain. Mortality was also recorded as it occurs and the experiment lasted for 12 weeks.

Fatty acid analysis

At the end of the experiment, five rabbits were randomly selected from each treatment for fatty acid analysis (FA). Meat (longissimus dorsi muscles) lipids from freeze dried, grounded samples were extracted with chloroform-methanol (2:1 v/v; Folch et al. (1957) with slight modification as described by Elshater et al. (2009). After extraction, FAs in the residual fat were esterified, using acid and base catalyzed methods as described by Elshater et al. (2009). Fatty acid methyl esters (FAMES) analysis was performed by gas chromatography mass spectrometry (GC-MS; Mussek-QM-2010 plus, China) equipped with electron impact (EI) detector. Separations of FAs were carried out on capillary column Model 7009 A, Punjab Technologies, India (30 m x 0.32 mm x 0.25 µm) using helium as carrier gas. Column temperature was held at 50°C for 1 min, and then the temperature was raised up to 150°C at the rate of 15°C per min. Temperature was later increased to 175°C at the rate of 2.50°C and hold for 5 min and finally increased to 220°C at the rate of 2.50°C per min and kept for 5 min. The identification of the peaks was made by comparison of the equivalent chain length with those of authentic fatty acid methyl esters. Peak areas were determined automatically using the Agilent gas chromatography chemstation software. The fatty acid concentrations were expressed in percentage of the sum of total identified peaks measured in each sample.

Table 1. Ingredient composition of experimental diets.

Ingredient	Quantity
Maize	36.00
Wheat offal	25.00
Palm kernel meal	20.20
Groundnut cake	10.00
Soya meal	5.10
Limestone	1.00
Bone meal	2.00
Lysine	0.10
Methionine	0.10
*Premix	0.25
Salt	0.25
Calculated analysis	(% DM)
Crude protein	16.44
Crude fibre	8.82
Ether extract	2.46
Calcium	1.34
Phosphorus	0.50
Energy (Kcal /kg)	2500.8

* Premix supplied per kg diet :- Vit A, 7,000 I.U; Vit E, 8 mg; Vit D3, 3000I.U, Vit K, 3 mg; Vit B2, 5.5 mg; Niacin, 25 mg ; Vit B12, 16 mg ; Choline chloride, 120 mg ; Mn, 5.2mg ; Zn, 25mg ; Cu, 2.6 g ; Folic acid, 2 mg ; Fe, 5 g ; Pantothenic acid, 10 mg ; Biotin, 30.5g ; Antioxidant, 56 mg.

Chemical analysis

Phytochemical analysis was carried out on the test material (ALO) using standard methods Sofowora (1993). Percentage composition of flavonoids, saponin, phytate, alkaloids, tannin and oxalate were carried out according to procedures outlined by Harbone (1984) and Boham and Kocipai-Abyazan (1974).

Statistical analysis

All data collected was subjected to one-way analysis of variance (ANOVA) using SPSS (23.0) and significant means will be separated using Duncan multiple range tests (Duncan, 1955) significant will be declared if $P \leq 0.05$.

RESULTS AND DISCUSSION

Table 1 reveals the nutrient content of the experimental diet, the chemical component contained crude protein (16.44%), crude fibre (8.82%), ether extract (2.46%), calcium (1.34%), phosphorus (0.50%) and metabolizable energy (2500.6 Kcal/kg). Higher crude fibre (12.08%) was reported by Salisu et al. (2012) when carrot leaf meal was included in the diets of growing rabbits. According to Alinnor and Oze (2011), adequate dietary intake of fibre can lower the risk of cardiovascular disease and improve digestion in animals. Similarly Unigwe et al. (2016) reported a crude protein of crude protein range of 15.83

to 16.38% in rabbits fed diets supplemented with dried neem leaf meal. However, all the values are within the nutritional requirement for rabbits as recommended by NRC (1977).

The phytochemical components of *A. lebbeck* seed in Table 2 reveals the presence of tannins, saponins, alkaloids, oxalate and glycosides. According to Hyun et al. (2018), a combination of multiple phytochemicals exerts synergistic effects to reduce negative effect of enteric infections. For instance, phenols have been reported to perform the role of antioxidants (Andjelkovic et al., 2016); tannins possess antimicrobial and antiviral activity (Adisa et al., 2010). Alkaloids and flavonoids have diuretic, antispasmodic, anti-inflammatory and analgesic effects (Ujowundu et al., 2010). According to Gadde et al. (2017), the main bioactive compounds of phytochemicals are polyphenols and their composition and concentration vary according to the plant, parts of the plant, harvesting season, geographical origin, environmental factors, storage conditions and processing techniques. However, the phytochemical values were below the lethal dose recommended by Alagbe and Grace (2019). This means that the feed is non-toxic and will not adversely affect the health of the animals; it further confirms the reports of Hawary et al. (2011) who stated that *A. lebbeck* oil contains minerals, vitamins, essential and non-essential amino acids, which makes it useful in livestock feeds.

Table 3 shows fatty acid profile of rabbit meat supplemented with different levels of *A. lebbeck* oil. Lauric acid (1.82 – 3.88%), myristic acid (2.00 – 3.22%), palmitic acid (13.8 – 22.9%), stearic acid (3.73 – 9.42%), arachidic acid (1.01 – 5.77%), behenic acid (0.29 – 0.95%), myristoleic acid (1.44 – 3.90%), palmitoleic acid (2.01 – 3.51%), linoleic acid (10.4 – 26.1%), oleic acid (1.00 – 2.11%), elaidic acid (0.80 – 0.87%), linolelaidic acid (15.1 – 28.7%), erucic acid (0.10 – 0.98%), eicosapentenoic acid (0.97 – 2.77%), α – linolenic acid (6.00 – 18.0%), arachidonic acid (3.11 – 7.22%), dihomo-gammalinolenic acid (0.77 – 1.30%), docosahexenoic acid (0.98 – 3.18%), total fatty acid ranges between (26.58 – 44.71%), total unsaturated fatty acid (41.47 – 77.87%), monounsaturated fatty acid (19.37 – 31.57%) and polyunsaturated fatty acid (22.37 – 46.30%) respectively. There were significant differences ($P < 0.05$) in the values obtained for TSFA, TUFA, MUFA and PUFA. TSFA value statistically reduced as the level of ALO increased in the diet. This confirms the earlier report by Alagbe et al. (2019) that ALO has the ability to reduce the cholesterol level in broiler chicks; this could be attributed to the presence of phytochemicals contained in ALO seeds. Hawary et al. (2011) also reported that ALO is rich in protein, essential and non-essential acid as well as PUFA. This is a clear indication that ALO has the ability to reduce SFA which in turn will improve the nutritive value of meat and reduce the incidence of cardiovascular infection. The result is consistent with the findings of Kamran et al. (2018) who reported that SFA level in the meat of rabbits decreased by feeding diets

Table 2. Phytochemical analysis of *Albizia lebbek* seed.

Parameters	% Composition	*Safe recommended level
Tannins	2.11	11.20
Saponin	6.33	7.02
Alkaloids	0.11	0.55
Oxalate	0.12	1.30
Glycosides	0.10	0.50

Source: Alagbe et al. (2019).

Table 3. Effect of *Albizia lebbek* seed oil on the fatty acids profile of rabbit meat.

Fatty acids	T1	T2	T3	T4	T5	SEM
C12:0	3.88 ^a	2.00 ^b	1.95 ^b	1.91 ^b	1.82 ^b	0.01
C14:0	3.22 ^a	2.62 ^b	2.40 ^b	2.29 ^b	2.00 ^c	0.18
C16:0	22.9 ^a	17.3 ^b	17.0 ^b	14.3 ^c	13.8 ^c	0.25
C18:0	9.42 ^a	8.71 ^a	6.61 ^b	3.00 ^c	3.73 ^c	0.15
C20:0	5.77 ^a	4.88 ^a	2.31 ^b	2.20 ^b	1.01 ^c	0.33
C22:0	0.95 ^a	0.44 ^b	0.37 ^b	0.30 ^b	0.29 ^b	0.02
C14:1c	1.44 ^c	3.08 ^a	3.71 ^a	3.88 ^a	3.90 ^a	0.12
C16:1c	2.01 ^b	2.21 ^b	2.93 ^b	3.18 ^a	3.51 ^a	0.03
C18:1c	10.4 ^c	19.0 ^b	19.2 ^b	25.8 ^a	26.1 ^a	0.74
C18:1n9t	1.00 ^c	1.72 ^b	1.88 ^b	1.93 ^b	2.11 ^a	0.02
C18:1n9c	0.82	0.86	0.80	0.83	0.87	0.17
C:22:1	0.10 ^b	0.67 ^a	0.71 ^a	0.76 ^a	0.98 ^a	0.02
C18:2n6	15.1 ^c	19.6 ^b	26.5 ^a	27.1 ^a	28.7 ^a	0.87
C20:5n3	0.97 ^c	1.60 ^b	1.72 ^b	1.88 ^b	2.77 ^a	0.10
C18:3n3	6.00 ^c	9.78 ^b	10.0 ^b	17.4 ^a	18.0 ^a	0.19
C20:4n6	3.11 ^b	3.40 ^b	3.59 ^b	3.80 ^b	7.22 ^a	0.22
C20:3n6	0.77 ^b	1.18 ^a	1.22 ^a	1.25 ^a	1.30 ^a	0.03
C22:6n3	0.98 ^c	2.05 ^b	2.10 ^b	2.18 ^b	3.18 ^a	0.42
TSFA ¹	46.14 ^a	35.95 ^b	30.64 ^b	24.00 ^c	22.65 ^c	0.03
TUFA ²	42.70 ^c	65.15 ^b	74.36 ^b	80.09 ^a	89.24 ^a	0.25
MUFA ³	15.77 ^c	27.54 ^b	29.23 ^b	30.08 ^b	35.07 ^a	0.44
PUFA ⁴	26.93 ^c	37.61 ^b	45.13 ^b	53.01 ^a	54.17 ^a	0.96
n-6:n-3 ⁵	1.38 ^b	1.80 ^b	2.41 ^a	2.89 ^a	3.65 ^a	0.03
Ant. Index ⁶	0.92 ^a	0.46 ^b	0.38 ^b	0.32 ^b	0.26 ^c	0.01

¹Total saturated fatty acid = C12:0 + C14:0 + C16:0 + C18:0 + C20:0 + C22:0

²Unsaturated fatty acid = (3 + 4)

³Mono unsaturated fatty acid = C14:1c + C16:1c + C18:1c + C18:1n9t + C18:1n9c + C22:1

⁴Polyunsaturated fatty acid = C18:2 n6 + C20:5 n3 + C18:3n3 + C20:4n6 + C20:3n6 + C: 22:6n3

⁵n-6: n-3 = (C18:2 n6 + C20:4n 6 + C20:3n 6 / (C20:5n3 + C18:3n 3 + C: 22 6n 3), ⁶Antherogenic index = (C12:0+ 4x(C14:0+ C16:0))/Σ of UFA.

enriched with flaxseed oil. PUFA and n-6: n-3 level increased as the level of ALO increased especially at 0.4 %, thus ALO has the ability to modulate the fatty acid of meat. According to Katalin and Loana (2017) omega-3 and omega-6 polyunsaturated fatty acids performs multiple biological roles such as influencing the inflammatory cascade, reducing oxidative stress, presenting neuro-protection and cardiovascular protection. This result is in agreement with the findings of

Suriya et al. (2014) and Trebusak et al. (2014) when linseed was supplemented in the diets of rabbits. However, omega-3 and omega-6 polyunsaturated fatty acid ratios were within the range recommended by Simopoulos (2001). Antherogenic index is a parameter used to determine meat safety, the lower the value the safer the meat. Supplementation of ALO at 0.4% significantly reduced the antherogenic index when compared with animals fed 0% ALO. This reveals that

ALO can reduce the incidence of arteriosclerosis and other cardiovascular infections which is in accordance with the reports of Kholif et al. (2017).

CONCLUSION

It can be concluded that ALO has proved their efficacy to perform multiple biological activities due to the presence of bioactive chemicals or secondary metabolites. Supplementation of ALO at 0.4% in the diet of rabbits modulates the fatty acid composition and nutritive value of meat. High PUFA composition in rabbit meat prevents cardiovascular and other degenerative diseases therefore bridging the gap between food safety and livestock production.

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