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# Modulating the Hemato-Biochemical Profile and Growth Efficiency of Post-Weaning Rabbits via *Clausena anisata* Essential Oil Supplementation

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## Article Information

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## Abstract

This research was undertaken to evaluate the effects of feeding weaner rabbits on diet supplemented with *Clausena anisata* essential oil at different levels. Forty clinically healthy weaned male rabbits about 5 to 6 month of age, with an average initial weight of  $720.0 \pm 0.71$  kg were randomly divided into four dietary groups with ten replicates each. The dietary treatments were: (A) basal diet without *Clausena anisata* oil; (B) basal diet supplemented with 0.3 mL *Clausena anisata* oil per kg DM feed daily; (C) basal diet supplemented with 0.6 mL *Clausena anisata* oil per kg DM feed daily; (D) basal diet supplemented with 0.9 mL *Clausena anisata* oil per kg DM feed daily in a completely randomized design for a duration of 60 days. Feed was provided ad libitum, and clean water was available at all times. Result obtained revealed that *Clausena anisata* oil increased ( $p < 0.05$ ) body weight gain, feed consumption as well as feed conversion ratio. High *Clausena anisata* oil treatment increased the concentration of pack cell volume, haemoglobin, red blood cell, white blood cell, lymphocytes and monocytes. *Clausena anisata* oil supplementation decreased ( $p < 0.05$ ) low density lipoprotein concentration and increase total protein, albumin, globulin, high density lipoprotein except urea, creatinine, alanine amino transferase and alanine amino transaminase ( $p > 0.05$ ). It was concluded that the supplementation of *Clausena anisata* essential oil up to 0.9 mL per kg DM daily improved weight gain, feed consumption and blood constituents of weaner rabbits without causing any deleterious effect on their health status.

## Introduction

Globally, there is a clear demand to reduce antibiotic use in poultry and in this way reduce the risk of antimicrobial resistance [1]. Antibiotics have been used to combat pathogens in the gut and by improving overall health in this way improve performance. However, their prophylactic use may induce bacterial resistance, environmental pollution and create toxic residues in their products [2]. The use of herbs has recently been accepted because of their therapeutic properties and are generally regarded as safe [3]. Beside organic acids, prebiotic, probiotics, essential oils have also been regarded as safe alternative to antibiotics. Essential oils contain full orchestra of bioactive compounds gentle in action over time and deeper connection to traditional medicine [4]. *Clausena anisata*, a pharmacy of rare phyto-compounds designed by nature to improve the vitality of the body [4]. It is an evergreen shrub belonging to the family Rutaceae which are widely distributed in Asia, Africa and some parts of Europe. It is also built for fever relief, dysentery, skin infection, digestion imbalance, tooth ache, sexually transmitted infection, arthritis amongst others [5].

*Clausena anisata* leaf, stem, fruits and root contains phenols, alkaloids, tannins, saponins and flavonoids known to possess antimicrobial, anti-diabetic, immune-stimulatory, antioxidant, anti-helminthic, cytotoxic, antidiarrheal, anti-inflammatory, gastro-protective, antifungal, cardio-protective, dermato-protective, anti-tumor, amongst others [6]. According to [7,8], chemical composition of *Clausena anisata* leaf essential oil contains (z) Anethole (34.24 %) and p-Cymene (16.01 %) as major compounds and have been reported to reduce inflammatory stress, protect blood vessels and fight free radicals, lower low-density lipoprotein, support cardiovascular health, modulate immune activity, reduce both acute and chronic infection and slower glucose absorption [9,10]. Previous studies by [11, 12] have shown that supplementation of essential oil in rabbits feeds may positively influence growth performance, nutrient absorption, secretion of digestive juices, gut microbiota and blood constituents. However, there are conflicting reports about their efficacy and appropriate dose in their diets. Some have been reported to induce toxicity or cytotoxic effect on cells at a high dose [13].

Therefore, some caution should be exercised in the use of essential oil as natural feed additives in rabbit diets.

This research is timely as it will further help to promote livestock sustainability, food safety and ascertain optimum dose for rabbits.

## Materials and Methods

### ❖ Experimental site

The experiment was undertaken at the Research Farm, Gandhi College of Agriculture, Rajasthan between July to October, 2017. The experimental site lies between latitude 8° 51'N and 9° 00'E and longitude 7° 00'N and 6° 08'E.

### ❖ Management of experimental animals, diet and design

Forty clinically healthy weaned male rabbits about 5 to 6 months of age, with an average initial weight of  $720.0 \pm 0.71$  kg, were used for the study. They were balanced by body weight and randomly assigned to one of four treatments in a completely randomized design for a duration of 60 days. Each treatment was replicated ten times with one rabbit per replicate. Two weeks before the commencement of the study, the pens and surrounding environment were properly cleaned, disinfected with Morigad plus Aquaclean (ratio 2:1) and well ventilated before the arrival of the rabbits. Management of animals was in accordance with the procedures outlined by Indian Institute of Animal Production. Rabbits were housed individually in wire cages (40 × 55 × 60 cm) under same environmental and management conditions. Basal diet (Table 1) was formulated in accordance with the National Research Council recommendation [14]. The dietary treatments were: (A) basal diet without *Clausena anisata* oil; (B) basal diet supplemented with 0.3 mL *Clausena anisata* oil per kg DM feed daily; (C) basal diet supplemented with 0.6 mL *Clausena anisata* oil per kg DM feed daily; (D) basal diet supplemented with 0.9 mL *Clausena anisata* oil per kg DM feed daily. The dosage of oil was added and manually mixed with feed before morning feeding to ensure full consumption. Feed was provided ad libitum, and clean water was available at all times. The proximate analyses of the diet samples were carried out according to [15]. Feed consumption was calculated by subtracting the weight of feed leftovers from the total amount provided. Cumulative body weight, body weight gain, and feed conversion ratio were determined from collected data throughout the study period.

### ❖ Collection of *Clausena anisata* leaves, extraction and analysis

*Clausena anisata* fresh leaves were harvested from Rajasthan India and sent to the department of botany, Gandhi College of Agriculture for identification before it was registered under voucher number JU/o8C/2017. The identified leaves were sorted and shade dried for 2 weeks until a constant weight was achieved. Dried *Clausena anisata* leaves were pulverized using

mechanical grinder. Extraction of oil was done by hydrodistillation with a Clevenger-type apparatus according to the procedures outlined by [15]. 250 g of the pulverized *Clausena anisata* was added to 1000 mL of water heated in a glass flask at 60 °C for 20 minutes, steam passes via the condenser and when cooled it was collected in a beaker. The oil collected by decantation at the end of the distillation was filtered, dried over column of anhydrous sodium sulfate, and introduced into glass bottles and stored in a refrigerator at 4 °C.

GC/MS analysis of *Clausena anisata* essential oil was done using Claudus 5006 GC-MS Auto Sampler (China) equipped with two silica capillary columns, interfaced with a quadrupole detector (single quadrupole acquisition Method-MS parameters report), source temperature 230°C, Quadrupole temperature 150 °C; the temperature program was 60 °C for 2 min, 60-240 °C at 3 °C/min, then kept at 240 °C during 8 min; injector temperature, 240 °C. The mass spectrometry transfer line temperature, 250 °C; carrier gas, helium at a flow rate of 0.7 ml/min; injection type, split, 20:1; ionization voltage, 70 eV; electron multiplier 1000 eV; scan range 33-400 amu; scan rate, 1.56 scan/s.

Identification of components Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology [16] having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library.

### ❖ Statistical analyses

All result data were subjected to a change study using the SPSS software package. Data were analyzed by one-way ANOVA. Also, significant differences among means were determined using Duncan's multiple comparisons. Range test at 0.05 level of significance.

**Table 1: Ingredient and chemical composition (% of DM)**

Ingredients	Quantity (%)
Maize	44.00
Wheat bran	20.00
Soy bean meal	28.00
Di-Calcium phosphate	7.00
DL-Methionine	0.20
L-Lysine	0.20
Growers Premix	0.25
Sodium Chloride	0.35
Total	100.0
<b>Chemical composition (%)</b>	
Dry matter	91.93
Organic matter	90.36
Crude protein	16.05

Crude fibre	12.87
Ash	9.64
Metabolizable energy	2607.4

Per 2.5 kg premix contains: Vit. A, 1600 IU; Vit.D<sub>3</sub>, 3000 IU; Vit. E, 15 mg; Vit. K<sub>3</sub>, 5 mg; Vit.B<sub>1</sub>, 3 mg; Vit.B<sub>2</sub>, 10 mg; Pantothenic acid, 12mg; Nicotinic acid, 25 mg; Folic acid, 1.5 mg; Biotin, 1.2 mg; Niacin, 60 mg; Vit.B<sub>6</sub>, 5 mg; Vit. B<sub>12</sub>, 25 mcg; Choline Chloride, 450 mg; Mn, 65 mg; Fe, 45 mg; Zn, 60 mg; I, 2 mg; Cu, 6 mg, and Se, 0.06mg

## Results

Major bioactive compounds in *Clausena anisata* essential oil by GC-MS analysis is presented in Table 2. The prominent bioactive compounds were;  $\alpha$ -Terpineol (18.21 %),  $\delta$ -Muuroleone (11.24 %), Anethole (10.90 %), p-Cymene (8.61 %),  $\alpha$ -Phallendrene (8.03 %), 7-Episesquithujene (7.92 %), Linalool (6.59 %), Germacrene B (6.27 %) and  $\alpha$ -Muuroleone (6.05 %).

Growth performance of weaned rabbits fed diet supplemented with *Clausena anisata* essential oil is presented in Table 3. Body weight gain was lowest ( $p < 0.05$ ) in treatment A (1155.9 g) than treatment B (1673.8 g), treatment C (1678.5 g) and treatment D (1882.4 g).

Table 4 shows the haematological indices of weaned rabbits fed diet supplemented with *Clausena anisata* essential oil. All the haematological parameters were within normal physiological ranges for healthy rabbits (Omokore and Alagbe, 2019).

Serum biochemical analysis of weaned rabbits fed diet supplemented with *Clausena anisata* essential oil is presented in Table 5.

## Discussion

Bioactive compounds have been previously associated with antimicrobial, anti-inflammatory, antioxidant and immune-stimulatory properties [18, 19, 20]. A synergy between these compounds relieves, heals, neutralize reactive oxygen species, stabilize cell membrane and improve the overall vitality of the body [21]. Foko et al. [17, 22] reported a higher level of p-Cymene (16.01 %) and Anethole (34.24 %) *Clausena anisata* essential oil compared with the outcome in this experiment. Geographical location, age of plant, storage period, processing method and specie have been reported to influence the composition of bioactive compounds in herbal plants [23, 24].

In the present experiment, the improved body weight gain may be due to enhanced digestion, nutrient absorption and modulation of enzymes in the gastro intestinal tract by bioactive compounds in *Clausena anisata* essential oil [25]. *Clausena anisata* essential oil contains 18.21 %  $\alpha$ -Terpineol, 11.24 %  $\delta$ -Muuroleone and 10.90 % Anethole and other triterpenoids which may be responsible for the increased weight gain because  $\alpha$ -Terpineol,  $\delta$ -Muuroleone and Anethole play important role in reducing harmful bacteria's in the digestive tract and maintaining a balanced gut environment [26]. This perhaps improved digestive efficiency [24]. Feed consumption was higher in diet B (6620.2 g), diet C (6528.5 g), diet D (6531.2 g) than diet A (5600.8 g) ( $p < 0.05$ ). *Clausena*

*anisata* essential oil influenced feed consumption, this result suggests enhanced feed flavor and palatability [27]. Similarly, it also appears that *Clausena anisata* essential oil enhanced better feed conversion ratio because of the presence of bioactive compounds. This is in line with the observation of [28] who noted improved feed intake and feed conversion ration with Eucalyptus oil supplementation to growing rabbits.

*Clausena anisata* essential oil supplementation increased the concentration of pack cell volume, haemoglobin and red blood cell suggesting oxygen sufficiency in the tissue to drive absorbed nutrient round the body, strengthened blood vessels and circulation, as well as absence of anemia and inflammation [30]. The high *Clausena anisata* essential oil supplementation increased white blood cell, leucocytes and monocytes suggesting improved immune activity by antibodies coordinating defense against oxidative damage [31]. As previously noted by [32], Linalool demonstrates potent capacity to neutralize the activities of free radicals and suppress the activities of inflammatory enzymes. This perhaps promoted antioxidant activity in the blood.

Serum biochemical analysis of weaned rabbits fed diet supplemented with *Clausena anisata* essential oil is presented in Table 5. *Clausena anisata* essential oil supplementation affected total protein, albumin and globulin concentration suggesting better protein reserve across the treatments. However, values obtained in this study were within the normal physiological ranges for healthy rabbits [33]. High density lipoprotein (HDL) value was lower ( $p < 0.05$ ) in treatment A (7.08 mg/dL) than treatment B (10.12 mg/dL), treatment C (10.53 mg/dL) and treatment D (11.72 mg/dL). HDL value was within the reference value cited by [34]. *Clausena anisata* essential oil supplementation decreased low density lipoprotein concentration, suggesting the absence of cardiovascular infection [35]. The high *Clausena anisata* essential oil supplementation did not affect ( $p > 0.05$ ) the concentrations of urea, creatinine, alanine amino transferase and alanine amino transaminase, indicating absence of renal and hepatic damage [36]. Urea, creatinine, alanine amino transferase and alanine amino transaminase were within the normal ranges 2.90 – 4.21 mg/dL, 0.55 – 1.55 mg/dL, 22.00 – 45.00 IU/L and 38.00 – 65.00 IU/L respectively reported by [37].

**Table 2: Major bioactive compounds in *Clausena anisata* essential oil by GC-MS analysis**

Compounds	Retention time (min)	% Area
Anethole	10.92	10.90
$\alpha$ -Terpineol	13.44	18.21
p-Cymene	19.16	8.61
$\delta$ -Muuroleone	20.80	11.24
$\alpha$ -Muuroleone	23.86	6.05
$\alpha$ -Phallendrene	28.77	8.03
7-Episesquithujene	30.65	7.92
Germacrene B	31.20	6.27
Linalool	32.08	6.59

GC-MS: Gas chromatography-mass spectrometry

**Table 3: Growth performance of weaned rabbits fed diet supplemented with *Clausena anisata* essential oil**

Parameters	A	B	C	D	SEM	P-value
Initial body weight (g)	720.71	719.29	720.11	718.8	2.76	0.51
Final body weight (g)	1876.7 <sup>c</sup>	2393.1 <sup>b</sup>	2398.7 <sup>b</sup>	2601.2 <sup>a</sup>	10.74	<0.001
Body weight gain (g)	1155.99 <sup>c</sup>	1673.81 <sup>b</sup>	1678.59 <sup>b</sup>	1882.4 <sup>a</sup>	8.65	<0.001
Total feed consumption (g)	5600.8 <sup>b</sup>	6620.2 <sup>a</sup>	6528.5 <sup>a</sup>	6531.2 <sup>a</sup>	28.44	0.108
Feed conversion ratio	4.84 <sup>a</sup>	4.00 <sup>b</sup>	4.00 <sup>b</sup>	3.88 <sup>c</sup>	0.03	<0.003

*Clausena anisata* essential oil supplementation increased the concentration of pack cell volume, haemoglobin and red blood cell suggesting oxygen sufficiency in the tissue to drive absorbed nutrient round the body, strengthened blood vessels and circulation, as well as absence of anemia and inflammation [30]. The high *Clausena anisata* essential oil supplementation increased white blood cell, leucocytes and monocytes suggesting improved immune activity by antibodies coordinating defense against oxidative damage [31]. As previously noted by [32], Linalool demonstrates potent capacity to neutralize the activities of free radicals and suppress the activities of inflammatory enzymes. This perhaps promoted antioxidant activity in the blood.

Serum biochemical analysis of weaned rabbits fed diet supplemented with *Clausena anisata* essential oil is presented in Table 5. *Clausena anisata* essential oil supplementation affected total protein, albumin and globulin concentration suggesting better protein reserve across the treatments. However, values obtained in t

Parameters	A	B	C	D	SEM	P-value
Pack cell volume (%)	27.16 <sup>b</sup>	34.96 <sup>a</sup>	34.18 <sup>a</sup>	35.66 <sup>a</sup>	0.96	0.116
Haemoglobin (g/L)	9.22 <sup>c</sup>	13.78 <sup>b</sup>	13.90 <sup>b</sup>	15.62 <sup>a</sup>	0.30	0.020
Red blood cell (106/ $\mu$ L)	1.81 <sup>b</sup>	2.55 <sup>a</sup>	2.51 <sup>a</sup>	2.59 <sup>a</sup>	0.12	0.010
White blood cell (103/ $\mu$ L)	8.23 <sup>b</sup>	10.71 <sup>a</sup>	10.88 <sup>a</sup>	11.95 <sup>a</sup>	0.20	0.165
Lymphocytes (%)	45.82 <sup>c</sup>	57.18 <sup>b</sup>	57.97 <sup>b</sup>	63.08 <sup>a</sup>	0.33	0.311
Monocytes (%)	10.09 <sup>b</sup>	13.12 <sup>a</sup>	13.64 <sup>a</sup>	14.06 <sup>a</sup>	0.25	0.213

<sup>a,b,c</sup> The different letters mean statistically significant at ( $p < 0.05$ );

**Table 5: Serum biochemical analysis of weaned rabbits fed diet supplemented with *Clausena anisata* essential oil**

Parameters	A	B	C	D	SEM	P-value
Total protein (g/dL)	4.89 <sup>b</sup>	5.23 <sup>a</sup>	5.28 <sup>a</sup>	5.53 <sup>a</sup>	0.04	0.010
Albumin (g/dL)	2.34 <sup>b</sup>	2.51 <sup>a</sup>	2.53 <sup>a</sup>	2.68 <sup>a</sup>	0.21	0.145
Globulin (g/dL)	2.55 <sup>b</sup>	2.72 <sup>a</sup>	2.75 <sup>a</sup>	2.85 <sup>a</sup>	0.18	0.102
HDL (mg/dL)	7.08 <sup>b</sup>	10.12 <sup>a</sup>	10.53 <sup>a</sup>	11.72 <sup>a</sup>	0.25	0.002
LDL (mg/dL)	24.61 <sup>a</sup>	18.56 <sup>b</sup>	18.32 <sup>b</sup>	16.09 <sup>b</sup>	0.20	0.001
Urea (mg/dL)	3.44	3.41	3.48	3.52	0.03	<0.001
Creatinine (mg/dL)	0.98	0.96	0.89	0.87	0.08	<0.001
Alanine amino transferase (IU/L)	27.19	28.61	28.79	28.94	0.30	0.366
Alanine amino transaminase (IU/L)	40.02	41.18	41.55	41.76	0.35	0.425

<sup>a,b,c</sup> The different letters mean statistically significant at ( $p < 0.05$ ); SEM: the standard error of mean

**Conflict of Interest: NIL**

**Funding Sources: NIL**

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#### Declarations:

#### Author's Contribution:

- Conceptualization, data collection, interpretation, drafting of the manuscript and intellectual revisions
- The author agrees to take responsibility for every facet of the work, making sure that any concerns about its integrity or veracity are thoroughly examined and addressed

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