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Supplementation of *Eucalyptus Camaldulensis* essential oils: Effects on haematology and serum biochemical indices of weaned pigs

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ABSTRACT

This experiment was undertaken to access the supplementation of *Eucalyptus camaldulensis* essential oils: effects on haemtology and serum biochemical indices of weaned pigs. A total of 80 cross bred pigs 6.88 ± 0.87 kg and weaned at 21 days of age were individually housed in pens and grouped based on their body weights into four treatments and fed basal diet with no *Eucalyptus camaldulensis* oil (ECO) in treatment 1 (T1), T2, T3 and T4 were fed diet supplemented with ECO at 200 mg, 400 mg and 600 mg/kg in a completely randomized design. Diets were sufficient in all nutrients according to National Research Council. Results on pack cell volume, haemoglobin, platelets, mean corpuscular volume, mean platelet volume, mean heamoglobin concentration and mean corpuscular haemoglobin concentrations, white blood cell, lymphocytes, basophils, monocytes and neutrophils were higher ($P<0.05$) in T3 and T4 than in T1 and T2. Alanine transaminase, aspartic transaminase, alanine phosphatase, urine and creatinine values were not influenced ($P<0.05$) by dietary supplementation of ECO while serum protein, albumin, globulin, cholesterol, bilirubin, glucose, phosphorus, sodium and chloride values were greater ($P<0.05$) in T3 and T4 relative to T1 and T2. However, all parameters were within the established ranges for healthy pigs. Results indicate that ECO can be supplemented up to 600 mg/kg in the diet of pigs without negatively affecting the health status of animals.

KEYWORDS

Eucalyptus camaldulensis essential oil; Haematology; Serum biochemical indices; Supplementation; Dietary supplementation; Haemoglobin

ARTICLE HISTORY

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Introduction

All around the world, there is growing interest in the current trend to reduce the prophylactic fortification of animal feed with antibiotics. Antibiotic supplementation, on the other hand, resulted in increased growth rates, reduced occurrence of various diseases, and better feed efficiency in the production of animals [1,2]. However, there is now mounting proof that prolonged, non-specific antibiotic exposure causes bacterial resistance, which has a considerable impact on the continued efficacy of these vital medications [3,4]. As a result, the prophylactic use of antibiotics in animal feed has been limited or prohibited in several nations. It becomes more urgent as a result to look for alternative methods of preserving animal performance and warding off diseases.

Essential oils are regarded as the most effective antibiotic substitute since they leave no trace, don't cause medication resistance, and don't have any negative side effects [5]. According to Melina and Borges, an essential oil is a liquid that comprises volatile components from plants with a complicated chemical makeup [6]. The leaves, seeds, flowers, roots, and other parts of the plant can all be used to extract them [5,7]. The active ingredients are present in essential oil-producing plants at amounts that can be extracted. Since essential oils are often extracted by steam distillation, the amount added to feed is little (less than 0.01%) compared to the amount needed for the same benefits when utilizing whole plants or plant components (more than 5%). Climate, harvest season, plant age, and cultivar within a plant variety are other factors that affect the content of essential oils. A wide range of biological or pharmacological actions, including anti-bacterial, antioxidant, antifungal, anti-inflammatory, hepatoprotective, antiseptic, and febrifuge, are expressed by medicinal plants like *Eucalyptus* camaldulensis [8]. It belongs to the family Myrtacea is a perennial evergreen plant in the genus Eucalyptus [9]. Over 900 species have been identified and spread across several regions of Africa, Europe, South America, and Asia [10,11]. Important minerals found in the plant, including salt, zinc, iron, magnesium, and potassium, play a critical role in activating enzymes that increase resistance to oxidative stress [12]. Traditional remedies for cough, cold, fever, arthritis, wound, sore, and gastrointestinal disorders include aqueous extracts from the bark, stem, and root [12]. Along with having therapeutic benefits, research has demonstrated that ethanolic extracts of bark, leaves, and roots can prevent the growth of several pathogenic organisms [9]. According to numerous studies, essential oils can positively impact a pig's performance, feed intake, antioxidant status, and overall health. However, there is a dearth of knowledge regarding eucalyptus oil's impact on animals. A prompt evaluation will provide information on the test material's degree of effectiveness and aid in filling the disparity between animal husbandry and food security. Therefore, this experiment was undertaken to access the supplementation of *Eucalyptus camaldulensis* essential oils: effects on haematology and serum biochemical indices of weaned pigs [13,14].

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Materials and method Experimental site and ethical guidelines

At the Sumitra Research Institute in Gujarat, India, that is located at 23o 13' N and 72o 41' E. This study was carried out in the Unit of Animal Nutrition and Biochemistry. All management practices were done following the ethical standards in India.

Processing of *Eucalyptus camaldulensis* essential oil using Clavenger apparatus

Fresh *Eucalyptus camaldulensis* leaf was harvested from Sumitra Research Institute premises in Gujarat in January 2023. Mature leaves are often preferred for essential oil extraction because they contain the highest concentration of essential oils compared to younger leaves. The leaves should be fully expanded, firm, and green in color, showing no signs of wilting or damage. For essential oil extraction, harvesting is typically done during mid-morning or early afternoon, after the dew has dried from the leaves but before the heat of the day causes oil evaporation. This time allows for optimal oil retention within the leaves. It was identified and authenticated by a certified taxonomist. The leaves were spread on a flat tray and separated manually from the stems and cut into smaller pieces with a kitchen knife. 100 grams of cut E. camaldulensis was transferred into 500 mL round bottom flask thereafter 300 mL distilled water was added to the round bottom flask. The clavenger apparatus was set up and the mixture in the round bottom flask was heated at 80°C for 60 minutes the steam produced passes through the condenser. Oil is collected in a beaker and stored in an air tight labeled tube.

Materials Required

Condenser, round bottom flask (500 mL), glass tubes, iron stand, burners, separatory funnel, glass tubes, condenser, beaker, stirrer and thermometer.

Livestock housing and management

For this test, 80 male crossbred pigs (Landrace, Yorkshire, and Duroc), weaned at 21 days, with an initial weight of 6.88 ± 0.87 kg, were purchased from a commercial breeding farm in India. At Sumitra Research Farm in Gujarat, pigs were placed in quarantine for fourteen days. They were given a basic diet and received Ivermectin preventive treatments (against parasites, following the maker's instructions) at (0.2 mL per kilogram). Before being divided into 5 semi-open-sided pens, each measuring 30 m by 18 m (length × breadth), the animals' weights were balanced before distributing them in their respective treatments. The trial lasted 56 days. Pigs were divided into four treatment groups, each of which had five replicates, each of which contained 20 pigs. The study was conducted using a completely randomized experimental design.

Experimental design

Experimental diets were sufficient in all nutrients recommended by NRC [15]. Diet 1: basal diet (Corn-soya meal) without E. camaldulensis essential oil; diet 2, 3 and 4 were supplemented with 200 mg, 400 mg and 600 mg E. camaldulensis essential oil /kg respectively, the selection of these doses (200 mg, 400 mg, and 600 mg/kg) is likely informed by prior research, safety considerations, expected biological effects, and practical applications in animal nutrition.

Measurements: Blood analysis

Blood for haematological studies was collected on the 56th day of the experiment from 10 randomly selected pigs per treatment. 2 mL of blood sample was collected very early in the morning from jugular vein of pigs into a sterile bottle with anticoagulant for haematological analysis using ADVIA 2120i automated analyzer (E6500 dual core, USA) with the following general specifications; Manual closed tube volume (175 µL), manual open tube volume (175 µL), automatic closed tube volume (175 µL) and work station specifications (intel pentium E6500 dual core 2.93 GHz 350 W desktop/tower), (3Gb/s 7200 RPM 16 MB Cache hard drive; 1 GB memory module CD-RW) and (17-inch torch screen colour LCD monitor).

Samples for serum biochemical investigation (2 mL) was collected into bottles free from anticoagulant using MISPA VIVA, true semi- automated clinical chemistry analyzer with the following technical specifications; Photometric Module (6 interference filters (EMF) 340, 405, 505, 546, 578 and 630 nm and 2 optional), silicon photo diode detectors, photometric range from 0 to 2.5 O.D, measuring module (30 µL flow cell volume), 10 mm square cuvette, Minimum aspiration volume – 300 µL and analysis mode (1-point linear and non-linear; 2-piont linear and non-linear; 1-point sample blank linear and non-linear; rate linear and non-linear; Immuno- turbidimetry).

Laboratory analysis of experimental diet

Analysis of experimental diet was carried out using Antaris TM FT-NIR analyzer with the following technical specifications; detectors (dual high performance ultra- cooled in GaAs extended range detectors), optical width (10.0 ± 0.3 nm actual FWHM), absorbance range (up to 3 AU), wavelength range (less than 0.02 nm to traceable standard reference material), wavelength precision (less than 0.005 nm), photometric noise full range (less than 20 µAu), ambient temperature (32 – 104°F) and ambient humidity (less than 95 % RH).

Statistics

All the statistical analysis was performed using SPSS (version 23.0). One way analysis of variance was used to determine statistical difference. P<0.05 was considered statistically significant.

Table 1. Experimental diets' gross composition

Materials	Quantity (kilogram)
Maize	50.00
Brewers dry grain	16.10
Soya meal	21.00
Fish meal (72 percent)	2.00
Bone meal	6.00
Oyster shell	3.00
Lysine	0.20
Methionine	0.15
Mineral/Vitamin Premix	0.25
Salt	0.30
Total	100.00
Calculated analysis (%)	
Crude protein	18.90

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Crude fibre	7.11
Ether extract	2.95
Calcium	1.61
Phosphorus	0.75
Energy (kcal/kg)	2700.7
Determined analysis (%)	
Crude protein	19.33
Crude fibre	8.20
Ether extract	3.05
Calcium	1.73
Phosphorus	0.84
Energy (kcal/kg)	2855.6

Mineral/Vitamin premix supplied per kg diet: - vitamin A, 8,000 I.U; vitamin E, 10.0 mg; vitamin D 4,000 I.U, vitamin K, 7.00mg; vitamin B2, 5.0mg; Niacin, 56 mg; vitamin B12, 25 mg; choline chloride, 100 mg; Manganese, 5.0 mg; Zinc, 35.1mg; Copper, 2.0g; folic acid, 2.5mg; Iron, 5.8g; pantothenic acid, 10mg; biotin, 30.5g; antioxidant,

Result and Discussion

Haematological parameters of weaned pigs fed diet supplemented with *Eucalyptus camaldulensis* essential oil

As shown in Table 2, pack cell volume, haemoglobin, mean platelet volume, red blood cell, mean corpuscular volume, mean corpuscular haemoglobin concentration (MCHCc) and mean corpuscular haemoglobin (MCHb) were altered by dietary supplementation of *Eucalyptus camaldulensis* essential oil in weaned pigs. The values were considerably (P 0.05) greater in animals fed diet 2, 3 and 4 compared to the control (diet 1). However, values were within were within the optimal range for healthy growing pigs cited by Rui et al. (2019) [16]. Increase in pack cell volume, haemoglobin, mean platelet volume and red blood cell is an indication of improved oxygen carrying capacity of the cells which translates to a better nutrient availability in the body of pigs [17]. Mean corpuscular volume, MCHb and MCHCc were within the normal ranges (31.04 – 70.05 fl), (18.51 – 52.00 pg) and (58.00 – 72.00 g/L) cited by Klem et al. [18]. These values attest to the fact that the pigs show no sign of anaemia or blood loss [19]. Supplementation of *Eucalyptus camaldulensis* essential oil in diet 3 and increased the white blood cell, neutrophils, basophils, monocytes and lymphocytes compared to the other groups (P 0.05). Values were within established ranges by Yeom et al., white blood cell play a key role in production of antibodies and resistance against diseases [20,21]. According to Afolabi et al., basophils have a role in the production of histamines and heparin in the bloodstream [22]. While lymphocytes and monocytes show an animal's capability for immunity, neutrophils are indicators of stress [23]. The improvement in blood parameters among pigs fed diet supplemented with *Eucalyptus camaldulensis* essential oil (ECO) could be linked to the presence some bioactive components in ECO especially α-pinene, β-pinene, eucalyptol amongst others [24,25]. These compounds have strong antimicrobial, antioxidant, anti-inflammatory, analgesics and hepato-protective properties [26]. Results recorded in this study are in consonance to the earlier reports of Farhadi et al. [27].

Table 2. Haematological parameters of weaned pigs fed diet supplemented with *Eucalyptus camaldulensis* essential oil

Parameters	1	2	3	4	SEM	Reference values
Pack cell volume (%)	28.55^b	35.10^a	35.88^a	36.00^a	1.03	27.10 – 38.00
Haemoglobin (g/L)	90.11^b	100.4^a	102.6^a	105.7^a	2.07	80.00 – 156.5
Platelet ($\times 10^9$/L)	116.88^b	130.00^a	142.55^a	145.06^a	9.46	100.0 – 340.7
Mean platelet volume (fl)	6.15^b	9.16^a	9.00^a	9.96^a	0.01	19.30 – 20.50
Red blood cell ($\times 10^{12}$/L)	7.11^b	9.88^a	9.97^a	10.6^a	0.03	6.84 – 13.00
Mean corpuscular volume (fl)	52.91^b	59.00^b	65.77^a	68.50^a	1.88	34.00 – 69.00
MCHb (pg)	30.80^b	40.11^a	44.13^a	48.92^a	1.01	19.00 – 50.00
MCHCc (g/L)	49.77^b	55.90^a	58.03^a	59.17^a	1.22	60.85 – 70.00
White blood cell ($\times 10^9$/L)	10.36^b	11.89^b	15.33^a	15.80^a	0.04	17.15 – 24.78
Neutrophils ($\times 10^9$/L)	8.40^b	8.55^b	11.00^a	11.59^a	0.01	10.70 – 12.53
Basophils ($\times 10^9$/L)	0.10^b	0.12^b	0.25^a	0.22^a	0.01	0.15 - 0.28
Monocytes ($\times 10^9$/L)	1.00^b	1.00^b	2.00^a	2.03^a	0.01	0.85 – 3.00
Lymphocytes ($\times 10^9$/L)	9.99^b	10.00^b	14.00^a	14.80^a	0.08	8.00 – 16.00

a,bMeans in a row without a similar superscripts differ (P<0.05); MCHb: mean corpuscular haemoglobin; MCHCc: mean corpuscular haemoglobin concentrations; SEM: standard error of mean; Reference values [28].

Serum biochemical indices of weaned pigs fed diet supplemented with *Eucalyptus camaldulensis* essential oil

As shown in Table 3, total protein, albumin and globulin values were higher (P<0.05) in T2, T3 and T4 than in other group (T1), the animals' liver and renal functions were stable and the diets were adequate in terms of nutrition [29]. All serum biochemical parameters examined in this study, were within the established ranges for healthy growing pigs reported by Petrovic et al., Cooper et al. [30,31]. Globulin helps to fight infection in the body of animals [32]. Total bilirubin, conjugated bilirubin and

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glucose levels in pigs in T3 and T4 were similar but lower than 5.80 – 11.00 μmol/L, 2.00 – 5.00 μmol/L and 2.00 – 6.00 mmol/L reported by Perri et al. [33]. Normal serum bilirubin indicates the absence of haemolysis and stable health status in animals [34]. Excessive serum glucose level is triggered during period stress or malnutrition [35]. Serum cholesterol, urea and creatinine values were comparable to the outcome of Friendship et al. [36]. Excessive concentration of urea and creatinine can cause liver and kidney damage while low levels could be due to low protein or severe liver damage [37]. Low cholesterol concentration among pigs fed diet supplemented with *Eucalyptus camaldulensis* essential oil eradicates the dangers of coronary disease [38]. Essential oils have also been reported to improve the shelf life of meat as well as boosting the level of polyunsaturated fatty acid [39]. Serum calcium, phosphorus, sodium and chloride were higher (P<0.05) in T3 and T4 than in other diets (T1 and T2) indicating effective enzymatic activities, maintenance of healthy blood volume and pressure, efficient control of the number of fluids in the body of animals [40].

Alanine phosphatase (ALP) was within the reference ranges for pigs [18]. Elevated alanine phosphatase indicates dysfunctional bile system in the body of animals. Similarly, values of Alanine Transaminase [AST] and Aspartate Transaminase [ALP] were not affected in all the groups (P>0.05). Though the supplementation of *Eucalyptus camaldulensis* essential oil reduced AST and ALP levels from T1 through T4, values were within the optimal ranges for healthy pigs suggesting efficient liver functions [40].

Table 3. Serum biochemical indices of weaned pigs fed diet supplemented with *Eucalyptus camaldulensis* essential oil

Parameters	1	2	3	4	SEM	Reference values
Total protein (g/dL)	6.89^{b}	7.92^{a}	8.12^{a}	8.15^{a}	0.01	3.00 – 9.53
Albumin (g/dL)	3.09^{b}	4.00^{a}	4.12^{a}	4.15^{a}	0.02	3.51 – 5.20
Globulin (g/dL)	3.80^{b}	3.92^{a}	4.00^{a}	4.00^{a}	0.01	3.00 – 7.00
Cholesterol (mmol/L)	4.17^{a}	2.00^{b}	2.02^{b}	2.17^{b}	0.06	2.31 – 6.51
Urea (mmol/L)	2.00	2.10	2.50	2.55	0.01	1.22 – 3.71
Creatinine (μmol/L)	49.80	49.88	50.00	51.10	1.09	34.10 – 96.17
Total bilirubin (μmol/L)	3.00^{b}	3.02^{b}	5.10^{a}	5.35^{a}	0.02	4.11 – 10.09
Direct bilirubin (μmol/L)	1.00^{b}	1.00^{b}	2.00^{a}	2.16^{a}	0.01	1.77 – 4.00
Glucose (mmol/L)	2.88^{b}	3.16^{b}	5.80^{a}	5.88^{a}	0.05	1.22 – 5.07
Calcium (mmol/L)	1.40^{c}	2.63^{b}	3.35^{a}	3.44^{a}	0.02	1.09 – 4.00
Phosphorus (mmol/L)	1.92^{b}	1.14^{b}	2.55^{a}	2.71^{a}	0.02	1.88 – 2.81
Sodium (mmol/L)	98.71^{b}	100.2^{a}	121.5^{a}	127.3^{a}	2.45	100.5 – 145.7
Chloride (mmol/L)	71.29^{b}	95.11^{a}	98.50^{a}	98.55^{a}	1.48	88.81 – 108.5
ALT (U/L)	72.10	71.44	70.06	75.22	2.06	34.00 – 81.21
AST (U/L)	65.60	68.01	63.80	67.29	2.81	27.10 – 71.34
ALP (U/L)	102.8	121.7	123.1	124.8	3.55	100.8 – 432.1

a,bMeans in a row without a similar superscripts differ (P<0.05); ALT: Alanine transaminase, AST: aspartate transaminase; ALP: alanine phosphatase; SEM: standard error of mean; Reference values [18].

Conclusions

In conclusion, ECO is rich in several phytonutrients or active compounds enabling it to offer antibacterial, antifungal, antioxidants, anti-protozoal, anti-inflammatory and immune-stimulatory properties. It can be supplemented up to 600 mg/kg in the diet of pigs without negatively affecting the health status of animals. They can also be used as an organic alternative to antibiotics to enhance the performance and productivity in livestock.

Disclosure Statement

No potential conflict of interest was reported by the author.

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