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Evaluating the impact of immobilized mixture of cinnamon, thyme, and oregano essential oils on performance of Chemballi ducks (*Anas platyrhynchos*)

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ABSTRACT

The study aimed to assess the effect of immobilized mixture of cinnamon, thyme and oregano essential oils (EOs) on the growth performance, immunity, behaviour and serum biochemical parameters of Chemballi ducks. Day old straight-run Chemballi ducklings (n=63) were divided into three treatment groups with three replicates per treatment with seven ducklings in each replicate. The immobilized mixture of EOs (cinnamon oil, thyme oil and oregano) was fed to the birds along with the basal diet as group T1 (control, without EOs), T2 (0.02% EOs) and T3 (0.04% EOs) respectively, from 2nd week onwards upto 8 weeks. The cumulative feed intake at the end of 8th week was higher (P<0.05) in T3 (0.04% EOs) followed by T2 (0.02% EOs) and T1 (control) group. The cumulative FCR was significantly (P<0.05) improved in 4th and 5th week with T2 having better FCR than T1 and T3. The T3 group showed higher cutaneous basophil hypersensitivity response (P<0.05) than control after 48 hrs however, no difference was seen at 24 hrs. The EOs had significant (P<0.01) effect on the feeding and resting behaviour of birds. The locomotor behaviour (walking and standing) was significantly (P<0.01) higher in T1 as compare to other treatment groups. Drinking behaviour, wing flapping, preening behaviour, tonic immobility, and serum biochemical parameters showed no significant differences across treatments. It is concluded that supplementation of immobilized mixture of cinnamon, thyme and oregano oils @ 0.04% in feed of Chemballi ducks positively influenced the growth, behaviour and immune response.

Keywords: Behaviour, Chemballi ducks, Essential oils, Growth, Immunity

Antimicrobial resistance (AMR) is a growing global concern primarily due to the overuse of antibiotics in livestock production, including poultry. The indiscriminate use of antibiotics has led to the emergence of resistant bacterial strains, posing significant threats to animal as well as human health. To address this challenge, alternatives such as essential oils (EOs) are gaining attention due to their antimicrobial, antioxidant, anti-inflammatory, and immune-modulatory properties (Donsì and Ferrari, 2016; Adaszynska-Skwirzynska and Szczerbinska, 2017; Han and Parker, 2017). Studies demonstrate that EOs not only improve poultry health and performance but also offer a sustainable, residue-free approach to combating AMR (Muthusamy *et al.* 2015; Paul *et al.* 2023).

EOs are complex mixtures of bioactive plant-derived compounds, including key active components such as carvacrol, thymol, cinnamaldehyde, and capsaicin, which have been widely incorporated into poultry diets for their multiple benefits (Burt, 2004; Lippens *et al.* 2005). Cinnamon essential oils and their components (cinnamaldehyde and eugenol) have been found to stimulant appetite and promote antioxidant, antibacterial and anti-inflammatory activities in poultry birds (Tabak *et al.* 1999). Thymol, a key component of thyme EO, is known for their antimicrobial, antioxidant, and anti-inflammatory properties, lipid metabolism and analgesic effects (Meeran *et al.* 2017; Luna *et al.* 2018). Similarly, oregano EO, composed primarily of carvacrol and thymol, exhibits strong biological activity that promotes poultry health and performance (Skoufos *et al.* 2016). Studies have reported enhanced body weight gain, improved feed efficiency and better immunity in poultry supplemented with EOs (Al-Kassie 2009; Ciftci *et al.* 2009; Perez-Roses *et al.* 2015; Hashemipour *et al.* 2013). While these benefits have been extensively documented in chickens, limited research exists on their application in ducks.

Ducks, next to chickens, play a crucial role in India's poultry industry. The indigenous breeds such as Chemballi duck, native to the Kuttanad region of Kerala are highly resilient and disease-resistant, making them ideal for sustainable poultry farming. However, the potential of EO supplementation, especially in immobilized form, remains largely unexplored in ducks. Immobilization

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enhances EO stability and ensures controlled release, optimizing its benefits in the digestive system. To our knowledge, this is the first study investigating immobilized EO supplementation in Chemballi ducks. This study aims to evaluate the effects of immobilized EO mixtures (cinnamon, thyme, and oregano) on growth performance, behaviour, immune response, and serum biochemistry in Chemballi ducks.

MATERIALS AND METHODS

Experimental design and dietary treatments: Sixty-three day-old straight-run Chemballi ducklings were randomly distributed into three groups (3 replicates/group) to nine pens, having seven ducklings each. They were reared in Duckery research unit of ICAR-Directorate of Poultry Research, Regional station, Bhubaneswar. The birds in group T1 were taken as control (diet devoid of EOs), T2 and T3 were supplied with the immobilized form of essential oil (cinnamon oil, thyme oil and oregano) @ 0.02% of feed and 0.04 % of feed, respectively, along with the basal diet. The experimental diet was fed to the birds from 2nd week onwards upto 8 weeks of age. The immobilized form of essential oils of cinnamon, thyme, and oregano was prepared as described by Paul *et al.* (2023).

Assessment of growth parameters: Individual body weights of the birds were measured at the end of each week and recorded. The weekly average body weights and weekly body weight gain were then calculated. Cumulative feed intake was determined by subtracting the remaining feed at the end of each week from the total feed provided to the birds during those weeks and FCR was computed accordingly.

Behavioural observations: Behaviour was recoded twice a week during the 4th, 5th and 6th week age. The behaviours were video recorded in 2 phases: morning (8:00 am - 9:00 am) and evening (3:00 pm - 4:00 pm). Behaviour was assessed as the proportion of birds performing the specific behaviour. The ethogram of different behaviours was recorded as per Table 1.

Immunological analysis: On 42nd day two birds from each replicate were immunized with 1% sheep red blood cells (SRBC) and the humoral mediated immunity was assessed using a microtitration haemagglutination method.

Table 1. Ethogram of different behaviors recorded in Chemballi duck

Ethogram	Description
Feeding	Head is directed towards the accessible feed supplies, while the beak positioned above or into the feeder seems to be taking the food.
Drinking	Beak above or within the drinker and seems to be taking the water.
Resting	Sitting, sleeping and laying or without doing any other behavior.
Walking	Progressing by taking one or more steps.
Standing	Abdomen is not touch with the litter and it is completely still without exhibiting any other behaviors.
Wing flapping	Simultaneously expanding both wings outward from the body and rapidly moving them up and down.
Preening	Beak-related behavior involves the bird's beak coming into contact with its own plumage.

The immunological response to SRBCs was assayed using the method developed by Siegel and Gros (1980), with a few modifications as described by Saxena *et al.* (1997). For measurement of T-cell mediated immune response, cutaneous basophilic hypersensitivity test was done at 49th day using phytohemagglutinin-P.

Serum samples two birds per replicate were collected at the end of the experiment for biochemical analysis using kits from Crest Biosystems, Coral Clinical Systems, Goa, India.

Data was evaluated using a one-way Analysis of Variance (ANOVA) with a Completely Randomized Design (CRD) in accordance with Snedecor and Cochran (1994). Duncan's Multiple Range Test (Duncan 1955) was used to determine the of level significance.

RESULTS AND DISCUSSION

Growth performance: The average body weight of day-old ducks was similar between the groups at the beginning of the experiment ranging from 103.33 g to 107.73 g. The body weight at 8th week significantly (P<0.05) different

Table 2. Effect of mixture of essential oil (Cinnamon, Thyme, and Oregano) supplementation on body weight (g) of Chemballi Ducks

Age (Week)	T_1	T_2	T_3	P value
1st	103.33±3.68	107.72±0.56	101.68±3.21	0.39
2nd	236.94±8.10	255.07±2.95	248.60±4.97	0.06
3rd	492.14±21.78	535.95±8.65	535.95±8.65	0.11
4th	643.66±11.43	674.55±2.66	638.19±21.16	0.08
5th	867.85±11.58	892.39±11.03	880.66±32.17	0.53
6th	1087.90^{b}±47.39	1168.18^{a}±6.44	1151.71^{a}±11.32	< 0.01
7th	1175.42^{b}±23.33	1238.59^{a}±12.03	1289.88^{a}±15.78	< 0.01
8th	1221.71^{b}±46.74	1323.17^{a}±13.23	1363.28^{a}±15.64	< 0.01

^{ab} Values bearing different superscripts in a row differ significantly (P<0.05).

Table 3. Effect of mixture of essential oil (cinnamon, thyme, and oregano) supplementation on feed intake (g) of Chemballi ducks

Age (Week)	T_1	T_2	T_3	P value
1st	164.13±5.51	168.8±6.58	172.30±5.87	0.66
2nd	433.80±8.03	438.46±7.10	467.47±9.58	0.06
3rd	1078.5^{b}±12.08	1094.5^{b}±10.56	1219.0^{a}±36.33	< 0.01
4th	1827.1^{b}±13.71	1886.8^{b}±20.47	2098.9^{a}±56.30	< 0.01
5th	2713.7^{b}±39.56	2805.4^{b}±36.4	3081.9^{a}±46.66	< 0.01
6th	3636^{b}±47.1	3727.6^{b}±28.5	4082.6^{a}±53.88	< 0.01
7th	4741.4^{b}±45.3	4858.1^{b}±34.04	5247.2^{a}±55.1	< 0.01
8th	5979.8^{c}±48.7	6137.3^{b}±36.37	6554.3^{a}±46.91	< 0.01

^{abc} Values bearing different superscripts in a row differ significantly (P<0.05)

among groups and ranged between 1221.71g to 1363.28g (Table 2). A significant increase in body weight was observed in the T2 and T3 group particularly in the 6th, 7th and 8th week. The increase in body weight observed in the groups (T2 and T3) supplemented with 0.02% and 0.04 % of the immobilized mixture of EOs corroborates with Al-Kassie (2009), Hasheimpour *et al.* (2013) and Ciftci *et al.* (2009) who reported increased body weight and improved feed efficiency on EO supplementation, particularly at higher doses. The positive correlation between EO supplementation and body weight gain suggests a potential role in improving nutrient utilization and feed efficiency. In the current study, immobilization might have aided in binding essential oil components to a carrier material, allowing for a gradual release over time similar to the effect of encapsulation of EOs suggested by Bosetti *et al.* (2020) and Ge *et al.* (2023). The prolonged exposure to essential oil compounds could contribute to the observed improvements in body weight. In contrast, Abildgaard *et al.* (2010) found detrimental effects on body weight gain due to variations in essential oil composition, concentrations and specific conditions of the experiments.

The cumulative feed intake was significant (P≤0.05) from 3rd week continuing up to end of 8th week (Table 3). However, no significant (P≥0.05) difference in cumulative feed intake observed between T1 and T2 up to 7th week. The increasing trend in feed intake with age aligns with the physiological growth patterns of ducks. The increased feed intake observed in current study aligns with the findings of Bosetti *et al.* (2020) indicating that supplementing mixture of EOs in poultry diets enhances palatability and stimulates appetite. Additionally, the immobilized form of the three essential oils (cinnamon, thyme, and oregano) may have facilitated a gradual release of bioactive components, potentially influencing taste, aroma, and overall palatability of feed. This sustained release might have led to prolonged and consistent appetite stimulation, contributing to the observed increase in feed consumption over time in the current study, and with to agreement observations made by Ogawa *et al.* (2020). Conversely, Hasheimpour *et al.* (2013), Amad *et al.* (2011), Halle *et al.* (2005) and Abildgaard *et al.* (2010) observed either a decrease or no significant change in feed intake with increasing essential oil doses.

The cumulative FCR increased with advancement of age from 2nd to 8th week in all the treatments reflecting the natural growth patterns and metabolic changes in the birds (Table 4). There was no significant (P>0.05) difference between the groups up to 3rd week. However, a notable divergence occurred in the 4th and 5th weeks, where the control (T1) and T2 group displayed significantly lower FCR suggesting positive impact of essential oil supplementation on feed efficiency during this specific period of duck growth. Contrary to these observations, from the 6th week to the 8th week, no significant differences in FCR were detected among the groups. The results suggest a nuanced

Table 4. Effect of mixture of essential oil (cinnamon, thyme, and oregano) supplementation on FCR of Chemballi ducks

Age (Week)	T_1	T_2	T_3	P value
1st	2.50±0.05	2.39±0.11	2.59±0.07	0.10
2nd	2.18±0.05	2.02±0.01	2.22±0.03	0.11
3rd	2.39±0.14	2.20±0.02	2.47±0.06	0.16
4th	3.02^{b}±0.08	2.96^{b}±0.02	3.50^{a}±0.14	0.01
5th	3.16^{b} ±0.10	3.27^{b}±0.06	3.28^{a}±0.07	0.06
6th	3.48±0.16	3.30±0.04	3.67±0.06	0.11
7th	4.17±0.09	4.05±0.03	4.19±0.04	0.25
8th	5.07±0.20	4.77±0.02	4.94±0.02	0.29

^{abc} Values bearing different superscripts in a row differ significantly (P<0.05).

Table 5. Effect of mixture of essential oil (cinnamon, thyme, and oregano) supplementation on behaviour of Chemballi ducks

Behaviours (proportion of birds)	T_1	T_2	T_3	P value
Feeding	38.43^{b}±0.76	41.38^{a}±0.64	42.40^{a} ±0.57	< 0.01
Drinking	47.50±0.98	44.66±0.74	45.17±1.58	0.21
Resting	27.21^{b}±1.10	28.57^{b}±1.15	32.42^{a}±0.60	< 0.01
Walking	18.17^{a}±0.74	16.20^{ab}±0.81	14.17^{b}±0.48	< 0.01
Standing	20.63^{a}±0.85	16.66^{b}±0.85	15.53^{b}±0.41	< 0.01
Wing flapping	5.89±0.54	6.23±0.54	6.8±0.61	0.53
Preening	8.95±0.59	9.86±0.46	9.86±0.52	0.39

[ab]Values bearing different superscripts in a row differ significantly (P<0.05)

impact of immobilized EO supplementation on FCR in Chemballi ducks and are in accordance to Bölükbasi *et al.* (2006) who noted an improvement in feed intake but did not observe an enhancement in FCR, potentially due to stagnation in body weight growth. In contrast, previous studies have reported better feed efficiency in broiler birds upon supplementation of mixture of EOs like thymol and carvacrol @ 200 mg/kg of the ration (Hasheimpour *et al.* 2013; Hasheimpour *et al.* 2014), cinnamon oil @ 500 ppm (Ciftci *et al.* 2009), oregano essential oils + vitamin C (Ghazi *et al.* 2015; Paul *et al.* 2023). The discrepancies observed in the results may be attributed to differences in the composition, dose, interactions in the digestive system and unique metabolic reactions of ducks in the present research warranting further studies.

Behaviour of birds: Significantly higher (P<0.01) proportion of birds engaged in feeding activities in both the EOs supplemented groups (T2 and T3) compared to the control group (Table 5). Further, higher (P<0.01) percentage of birds showed resting behaviour in T3 group. The locomotor activities (walking and standing) were significantly higher (P<0.01) in control group. However, drinking behaviour, wing flapping, preening behaviour and average duration in tonic immobility test did not vary across treatments.

The enhanced feeding behaviour observed in EO supplemented groups may be attributed to the stimulatory effect of immobilized EOs, suggesting increased palatability or appetite stimulation, as reported by Ogawa *et al.* (2020) and Nguyen *et al.* (2023). Additionally, the calming effect may have contributed to increased feed intake, further promoting feeding activity. The significantly higher resting behaviour in birds supplemented with a higher dose (0.04%) of immobilized EOs aligns with Ramadan (2013), indicating that EOs promote rest, while Sohrabi *et al.* (2017) also reported a calming effect of cinnamon, thyme, and oregano EOs on birds. Higher locomotor behaviour (walking and standing) evidenced in T1 group corroborates with Symeon *et al.* (2010 and 2014) who found that EOs drastically reduced the movement of birds and the effect was more pronounced at higher inclusion levels. Overall, the behavioural changes observed in the present study could be attributed to the potential stress reducing properties of the EOs (cinnamon, thyme and oregano) on the birds.

Immunity status: The observed HA titers ranged from 5.5 to 5.83 and notably, there was no significant difference among the groups (Table 6). This suggests a relatively consistent humoral immune response across the experimental conditions. Previous studies have reported enhanced immunological responses to vaccines in broiler chickens upon administration of Eos (Sadeghi *et al.* 2012' Dey *et al.* 2023; Du *et al.* 2016). The contrasting results obtained in the present study might be due to specific composition of the oils used and the genetics of the duck population warranting future research application of EOs for maximum immunomodulatory effects in ducks.

The cutaneous basophil hypersensitivity (CBH) response was used to assess cellular immunity, which involves the activation of phagocytes and T-lymphocytes. CBH response ranged 96.51-101.22 and 107.04-144.31 at 24 and 48 hr, respectively. There was no significant difference in CBH response after 24 hr. Whereas, a significant (P<0.05) difference was observed in T3 (0.04% EO) than T1 (control) after 48 hr. Hashemipour *et al.* (2013) and Halas *et al.* (2011) observed improved cellular immune responses in mice that were given EOs as supplements. The results indicate that the immobilized EOs of cinnamon, thyme and oregano possess the capacity to enhance cellular immunity,

Table 6. Effect of mixture of essential oil (cinnamon, thyme, and oregano) supplementation on HA titre and CBH response of Chemballi ducks (7^{th} week)

Parameters	T_1	T_2	T_3	P value
CBH (24 hr)	96.51±5.04	99.59±4.48	101.22±5.68	0.80
CBH (48 hr)	107.04^{b}±9.21	129.54^{ab}±8.56	144.31^{a}±9.93	0.04
HA (log_2)	5.5±0.42	5.5±0.42	5.83±0.4	0.813

[ab]Values bearing different superscripts in a row differ significantly (P<0.05).

Table 6. Effect of mixture of essential oil (cinnamon, thyme, and oregano) supplementation on serum biochemical parameters of Chemballi ducks (7th week)

Parameters	T_1	T_2	T_3	P value
Glucose (mg/dl)	141.39±3.88	143.15±5.84	141.69±4.39	0.96
Total Protein (g/dl)	3.20±0.03	3.26±0.04	3.31±0.03	0.90
Albumin (g/dl)	1.63±0.03	1.65±0.04	1.67±0.04	0.79
Globulin (g/dl)	1.56±0.05	1.61±0.04	1.63±0.02	0.44
A/G ratio	1.05±0.05	1.08±0.05	1.17±0.06	0.28
Triglyceride (mg/dl)	25.28±1.03	25.52±1.09	25.63±0.97	0.97
Cholesterol (mg/dl)	116.62±3.11	118.52±3.96	113.82±1.78	0.57
AST (U/dl)	161.82±3.02	162.63±5.06	166.06±4.14	0.75
ALT (U/dl)	53.00±1.30	55.07±1.41	53.62±1.31	0.55

especially when used at higher concentrations.

The effects on both humoral and cellular immunity seen in the present study can be linked to the immunomodulatory capabilities (Adaszynska-Skwirzynska and Szczerbinska, 2017; Donsì and Ferrari, 2016) of particular chemicals found in the EOs. The polyphenol component of carvacrol and thymol, emphasized by Perez-Roses *et al.* (2015), might have a vital function in enhancing the immune response.

Serum biochemical profile: The supplementation of immobilized EOs (cinnamon, thyme and oregano) had no significant effect on serum glucose, total protein, albumin, globulin, A/G ratio, triglyceride, cholesterol, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels in ducks (Table 6). The present findings corroborate with Abo Ghanima *et al.* 2020, who observed non-significant effects on total cholesterol, triglycerides, glucose, total protein, albumin, globulin, ALT and AST while using oregano EO in chickens. In contrast supplementation of thyme oil and fennel oil significantly increased levels of total protein, albumin, cholesterol, triglycerides and bilirubin but decreased glucose level in growing ducks (Gulec *et al*. 2013). Previous studies have reported improved liver functions, protein levels and AST levels in broilers supplemented with essential oils like cinnamic aldehyde, thyme and carvacrol mixture (Reis *et al*. 2018), cinnamon oil (Saied *et al.* 2022), thymol and carvacrol (Hashemipour *et al*. 2014), respectively. The discrepancies in the serum profile may be attributed to variations in EO types, concentrations, species difference and study durations. Additionally, individual variations in species responses to EO supplementation, as well as differences in basal diets, could have contributed to the observed differences.

The results of present study indicate that dietary supplementation of immobilized mixture of cinnamon, thyme and oregano essential oils @ 0.04% improved the growth performance, behaviour and immune response in Chemballi ducks without any adverse effect. This study underscores EOs with a promising alternative over antibiotic resistance in poultry. Further studies may be envisaged to assess the effects of these oils in commercial poultry including ducks for a sustainable and antibiotic free production.

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