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Effect of Dietary Incorporation of Lemongrass (*Cymbopogon citratus*) Essential Oil on the Nutrient Metabolism, Carcass Characteristics and Gut Microbes of Broilers

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ABSTRACT

The experiment was conducted from July-September, 2024 at the Poultry unit of the College of Veterinary and Animal Science, Navania, Vallabhnagar, Udaipur (Rajasthan), India to study the effect of dietary inclusion of the herbal feed additive lemongrass (*Cymbopogon citratus*) essential oil on nutrient metabolism, carcass characteristics, and gut microbes as an alternative to antibiotic feed additives. A six-week experimental trial was conducted on ninety-six day-old (VENCOBB-400) broiler chicks, which were divided into four treatment groups. The chicks were fed a broiler basal diet in the T_1 group, a broiler basal diet with 3 ml kg^{-1} lemongrass essential oil in the T_2 group, a broiler basal diet with 3.5 ml kg^{-1} lemongrass essential oil in the T_3 group, and a broiler basal diet with 4 ml kg^{-1} lemongrass essential oil in the T_4 group. During the last week of the six-week trial period, two birds per replicate under each treatment were randomly selected and shifted to metabolic cages to conduct a nutrient metabolism trial. On the 42nd day, birds were sacrificed for carcass study and gut microbial assay. The results showed substantial improvements (p<0.05) in the metabolism of dry matter, crude protein, nitrogen-free extract, and crude fiber, while the metabolism of ether extract and total ash showed non-significant changes (p>0.05). The percentage of eviscerated weight increased significantly (p<0.05), while the percentage of dressing weight and giblets weight varied non-significantly (p>0.05). Additionally, there was a highly significant (p<0.01) reduction in E. coli and total viable count.

KEYWORDS: Broiler, lemongrass, essential oil, nutrient metabolism, carcass, microbiota

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1. INTRODUCTION

Chicken meat and eggs are the poultry products most often consumed, they are the most popular choice among the general public since it is inexpensive, versatile in a variety of cuisines, and widely available. Chicken meat is currently one of the fastest-expanding segments of India's agricultural economy. Consumers are becoming more concerned about animal welfare, the environment, and health, and poultry has the lowest carbon footprint and price compared to other types of meat, as well as a healthy combination of protein and low fat (Anonymous, 2023).

To improve poultry health and growth antimicrobials have traditionally been used. However, as the risk of developing antibiotic-resistant infections has grown, antibiotics for therapeutic and preventative purposes in food animals are gradually being phased out (Ricke et al., 2020). The research should aim at developing effective alternatives to antibiotics to maintain the efficiency of present poultry production while minimizing food safety concerns. Phytogenic feed additives are one of the most promising alternatives. Phytogenic feed additives are plant-derived ingredients used in chicken diets to improve performance and quality (Ebrahim et al., 2020). Fibers and active phytochemicals such as terpenoids, flavonoids, lignans, sulfides, plant sterols, polyphenolics, coumarins, carotenoids, and saponins, as well as essential oils, are examples of phytogenic feed additives (Kiani et al., 2022).

Lemongrass (*Cymbopogon citratus*) is an aromatic tropical perennial herb with long, thin leaves that can reach a height of 3.5 meters (Parade et al., 2019). It is grown for essential oil in Asia, South America, and Africa's tropical and subtropical climates (Shah et al., 2011). Lemongrass (*Cymbopogon citratus*) essential oil is one of the essential oils used as feed supplements to improve poultry growth and performance (Peter and Babu, 2012). Lemongrass essential oil accounts for around 1–2% of the plant's dry weight (Ranade, 2015). Due to the presence of higher content of aldehyde it possesses lemony odor (Sehajpal et al., 2023). Lemongrass essential oil contains citral (a combination of geranial and neral), which gives various pharmacological effects, including antifungal, antibacterial, antiviral, anticancer, and antioxidant capabilities (Mukarram et al., 2021). Additionally, lemongrass essential oil contains limonene, an anti-inflammatory molecule (De Souza et al., 2019).

The gut health of broiler chickens is impacted by bacterial contamination in the gastrointestinal tract, especially gram-negative bacteria. Common harmful Gram-negative bacteria that disrupt the gut microbiota and reduce broiler productivity include Salmonella species and E. coli (Hasan-Al-Sharif et al., 2023). Lemongrass essential oil's bioactive components enhance immunity, function as antioxidants, and are anti-inflammatory, aiding in liver damage healing by suppressing reactive oxygen species, lipid peroxidation, pro-inflammatory mediator release, and cellular permeability (Rahman et al., 2022). In addition to antimicrobial and antioxidant activity, essential oils improves nutritional absorption (Banjo et al., 2022), it increases insulin sensitivity and raises metabolic rate and have digestive stimulant properties (Srivastava, 2021). The Lemongrass essential oil promotes intestinal enzyme activity (Alagawany et al., 2021). Optimal enzyme production is necessary for optimal feed digestion and nutrient absorption in the colon, this reflects on production performance such as carcass characteristics (Al-Azzami and Mohammed, 2023).

Improved nutrition metabolism, along with a reduction in intestinal bacteria, will result in better growth performance in broiler chickens, and carcass quality influences their long-term marketability. Therefore, the objective of the study was to determine how adding lemongrass (*Cymbopogon citratus*) essential oil to the diet as a substitute for antibiotic feed additives would impact nutrient metabolism, carcass characteristics and intestinal microbiota.

2. MATERIALS AND METHODS

2.1. Study sites

The experiment was conducted during *kharif* (July-September, 2024) at poultry unit (24.65, 74.02) of College of Veterinary and Animal Science, Navania, Vallabhnagar, Udaipur (Rajasthan), India to evaluate the effect of varying levels of lemongrass (*Cymbopogon citratus*) essential oil on ninety-six (96) unsexed, apparently healthy, day-old ('VENCOBB-400') commercial broiler chicks of the same hatch purchase.

2.2. The experimental design

The chicks were put into four treatment groups; T_1, T_2, T_3, and T_4, with three replicas; R_1, R_2 and R_3, having eight birds each. The chicks were fed a broiler basal diet in the T_1 group, a broiler basal diet added with @ 3 ml kg^{-1} lemongrass essential oil in the T_2 group, a broiler basal diet with @ 3.5 ml kg^{-1} lemongrass essential oil in the T_3 group, and a broiler basal diet with @ 4 ml kg^{-1} lemongrass essential oil in the T_4 group.

2.3. Experimental feed and proximate composition

As per Anonymous (2007), from the commercial suppliers, the basal broiler feed and lemongrass essential oil produced by steam distillation were purchased. Following Anonymous (2016), the proximate composition of the basal feed (Table 1) was examined. At the included levels of lemongrass essential oil, it had a non-significant effect on the feed's proximate composition.

Table 1: Proximate composition of the broiler pre-starter, starter, and finisher feed (% DM basis)

S. No.	Chemical composition	Broiler pre-starter	Broiler starter	Broiler finisher
1.	Dry matter	90.24	91.09	91.28
2.	Crude protein	23.03	22.23	20.54
3.	Ether extract	4.36	4.53	4.87
4.	Crude fibre	4.25	4.30	4.45
5.	Total ash	5.57	5.93	6.26
6.	Nitrogen free extract	62.79	63.01	63.88

2.4. Methods of data collection

Following the six weeks trial, on the last week two birds/ replicate under each treatment were randomly selected and shifted to metabolic cages. During metabolic trial the birds were separately fed with respective treatment diets. The one week metabolic trial included two days adaptation period followed by five days collection period. During the collection period samples of feed, residue and excreta by an individual bird were stored and subjected to proximate analysis as per Anonymous (2016). The following formula was used to determine the diet's treatment-wise dry matter metabolism (%):

$$\text{Dry matter metabolism (\%)} = \frac{(A)-(B)}{(A)} \times 100$$

Where: A=Weight of the dry matter consumed (g)

B=Dried weight of the excreta voided (g)

Similar formula was used to determine the metabolism (%) of crude protein, ether extract, crude fiber, nitrogen-free extract (NFE), and total ash.

The carcass characteristics of broiler chickens were analyzed on the 42^{nd} day. Before sacrifice, each chosen birds were weighed and allowed to fast for 12 hours to clear its stomach. The chosen bird's weights were comparable to the average live weight of the corresponding populations. The ileum content were collected in the sterile sampling tubes for the microbial assay.

After the jugular vein was cut for slaughter, each bird were allowed to bleed for five minutes. The dressed weight was stated as a percentage of the live weight just before they were sacrificed. After making a median cut in the abdomen, the dressed bird's trachea, crop, and viscera were removed. The lungs were removed with scraps. The gastrointestinal tract was separated from the heart, liver, pancreas, spleen, and gizzard. To measure the eviscerated weight and express it as a percentage of the pre-slaughter weight, the giblets (heart, liver, and gizzard) were cleaned and giblets weiht percentage were calculated.

Enumeration of the microbial population in the ileum content were used for serial dilution. Then inoculated on EMB (Eosin-Methylene Blue) and nutrient agar for the estimation of total E. coli and total vibal count, respectively and incubated at 37°C for 18–24 hours. Discrete colonies on the plate were counted using the colonies counter and estimated in log10 cfu/ml.

2.5. Statistical analysis

Data from the experiment related to the effect of lemongrass essential oil were statistically analyzed using a completely randomized design per Snedecor and Cochran (1994), and the significance of mean differences was assessed using Duncan's New Multiple Range Test (DNMRT), which was modified by Kramer (1957).

3. RESULTS AND DISCUSSION

3.1. Nutrient metabolism trial

The average daily feed intake and faeces voided by the chickens during metabolic trail were shown in the Table 2.

Table 2: The average daily feed intake and faeces voided by the chickens during metabolic trial

Treatment groups	Feed intake (g)*	Faeces voided (g)*
T_1	147.49	101.64
T_2	144.14	98.42
T_3	142.11	96.17
T_4	137.77	90.64

*Weight with moisture

The data of nutrient metabolism (%) for different treatment groups are presented in Table 3. The overall mean values of dry matter metabolism (%) were 73.27, 74.01, 74.07, and 74.86 in the T_1, T_2, T_3 and T_4 treatment groups, respectively. Statistical data analysis had revealed a significant ($p<0.05$) effect of lemongrass essential oil on dry matter metabolism in broiler chickens. The overall mean values of crude protein metabolism (%) were 67.49, 70.96, 70.12, and 72.59 in the T_1, T_2, T_3 and T_4 treatment groups. Statistical data analysis had revealed a significant ($p<0.05$) effect of supplementation of lemongrass essential oil on crude protein metabolism.

The overall mean values of ether extract metabolism (%) were 75.91, 77.28, 76.88, and 79.18 in the T_1 (control), T_2, T_3 and T_4 treatment groups, respectively. Statistical analysis of data on ether extract metabolism had revealed non-significant ($p>0.05$) differences among the control and various treatment groups.

The overall mean values of crude fiber metabolism (%) were 45.91, 44.03, 43.43, and 41.99 in T_1 (control), T_2, T_3 and T_4 treatment groups, respectively. Statistical data analysis

Table 3: Effect of lemongrass essential oil on metabolism (%) of nutrients in broiler chickens

Metabolism (%)	Treatment groups				**/*/N.S
	T_1	T_2	T_3	T_4	
Dry matter	73.27^b	74.01^{ab}	74.07^{ab}	74.86^a	*S
Crude protein	67.49^b	71.29^a	70.12^{ab}	72.59^a	*S
Ether extract	75.91	77.28	76.88	79.18	N.S
Crude fibre	45.91^a	44.03^{ab}	43.43^b	41.99^b	*S
Total ash	51.23	51.83	52.97	53.86	N.S
Nitrogen free extract	77.10^b	81.02^a	82.20^a	78.30^{ab}	*S

Means with different superscripts in a row differ significantly *S: Significant at 5% probability ($p<0.05$); **S: Significant at 1% probability ($p<0.01$); NS: Non-significant ($p>0.05$)

had revealed a significant ($p<0.05$) decrease in crude fiber metabolism due to lemongrass essential oil.

The overall mean values of total ash (TA) metabolism (%) were 51.23, 51.83, 52.97, and 53.86 in the T_1 (control), T_2, T_3, and T_4 treatment groups, respectively. Statistical analysis of variance had revealed a non-significant ($p>0.05$) effect of lemongrass essential oil on total ash metabolism in broiler chickens.

The overall mean values of nitrogen-free extract metabolism (%) were 77.10, 81.02, 82.19, and 78.29 in T_1 (control), T_2, T_3, and T_4 treatment groups, respectively. Statistical analysis of data on nitrogen-free extract metabolism had revealed a significant ($p<0.05$) difference between the control and various treatment groups.

The findings of dry matter, crude protein, crude fiber, and nitrogen-free extract metabolism were in line with Banjo et al. (2022), who had observed a significant improvement in their metabolism. However, the results for ether extract were consistent with Samant (2019), who had found a non-significant result. Giridharrao (2015) had observed a significant difference in the metabolism of crude protein.

In contrast, the present findings for dry matter and crude protein differed from those of Samant (2019), who had observed a non-significant difference in the metabolism of dry matter and crude protein, though quantitative improvement was observed in the lemongrass oil and turmeric rhizome powder treated groups.

The present findings of ether extract and total ash differed from those of Banjo et al. (2022), which had shown a significant difference. Lemongrass contains citral that helps to digest food, while polyphenols increase the use of energy and enhance protein, fat, and ash digestion. It can also promote nutrition absorption (Banjo et al., 2022). The interactions of phenolic compounds with proteins may lead to changes in the physicochemical properties of proteins such as solubility, thermal stability, and digestibility (Labuckas et al., 2008 and Rawel et al., 2001).

3.2. Carcass characteristics

The data for average dressing percentages (Table 4) were 75.75, 78.96, 79.19, and 77.99 (%) in the T_1, T_2, T_3 and T_4 groups, respectively. Statistical analysis of the data had revealed a non-significant ($p>0.05$) difference in the average dressing percentages between the groups.

Table 4: Effect of lemongrass essential oil on carcass evaluation parameters (% of live weight) of broiler chickens

Carcass parameters (Weight %)	Treatment groups				S**/S*/N.S
	T_1	T_2	T_3	T_4	
Dressed	75.75	78.96	79.19	77.99	N.S
Eviscerated	68.97^b	70.80^{ab}	71.50^a	70.22^{ab}	*S
Liver	1.79	1.86	1.83	1.79	N.S
Heart	0.61	0.61	0.64	0.62	N.S
Gizzard	1.53	1.53	1.46	1.50	N.S
Giblet	3.94	4.00	3.94	3.91	N.S

Means with different superscripts in a row differ significantly *S: Significant at 5% probability ($p<0.05$); **S: Significant at 1% probability ($p<0.01$); NS: Non-significant ($p>0.05$)

The average eviscerated weight percentages were 68.97, 70.80, 71.50, and 70.22 (%) in the T_1, T_2, T_3 and T_4 groups, respectively. Lemongrass essential oil had had a significant ($p<0.05$) influence on the eviscerated weight percentages of broiler chickens.

The data for average liver weight (%), heart weight (%), gizzard weight (%), and giblet weight (%) were found to be 1.79, 0.61, 1.53, and 3.94 in the T_1 group; 1.86, 0.61, 1.53, and 4.00 in the T_2 group; 1.83, 0.64, 1.46, and 3.94 in the T_3 group; and 1.79, 0.62, 1.50 and 3.91 in the T_4 group, respectively. Statistical analysis of the data had revealed that the results for organ (heart, liver, gizzard, and giblets) weight percentage (%) to live body weight of broiler chickens differed non-significantly ($p>0.05$).

The results on carcass characteristics were consistent with some earlier research, such as Samant (2019), who had incorporated lemongrass oil and turmeric rhizome powder but did not find a significant ($p>0.05$) difference in the dressing percentage without giblets and the percentage with giblets. Ghanima et al. (2021) and Khalifah et al. (2021) had revealed that except for carcass percentage, which did not differ significantly ($p>0.05$) after dietary supplementation with lemongrass essential oil, other carcass parameters had shown a significant change ($p<0.01$). Alagawany et al. (2021) had shown non-significant effects ($p>0.05$) of dietary lemongrass essential oil on the percentages of the carcass, heart, gizzard, liver, giblets, and dressing for the pre-slaughter weight.

However, these findings varied from those of Alagbe (2020), who had shown that for the carcass weights, birds fed a diet supplemented with LGO had performed better (p<0.05) than birds on the control diet, and liver, heart, lungs, spleen, gizzard, and intestine weights had differed significantly (p<0.05) among the treatments. Srivastava (2021) had indicated that lemongrass and peppermint essential oils in combination had significantly (p<0.05) improved dressed yield with and without giblets, weights of heart, gizzard, and giblets. Al-Azzami and Mohammed (2023) had found that lemongrass-treated groups had excelled in dressing percentage with edible giblets and without edible giblets.

3.3. Gut microbial assay

The average E. coli counts in replicas of the T_1, T_2, T_3, and T_4 groups were 5.94, 5.52, 5.44, and 5.05 log10 cfu ml^{-1} caecal samples, respectively. The difference was statistically highly significant (p<0.01).

The average viable counts in replicas of the T_1, T_2, T_3, and T4 groups were 8.32, 8.13, 8.06, and 8.01 log 10 cfu ml^{-1} caecal samples, respectively. The total viable counts between the groups were statistically highly significant (p<0.01).

Earlier findings on lemongrass supplementation were consistent with those of Alagawany et al. (2021), who found that groups supplemented with lemongrass essential oil exhibited lower coliform, *E. coli*, and Salmonella colonization (p<0.01) than those in the control group. Khalifah et al. (2021) revealed that dietary lemongrass essential oil supplementation tended to decrease the concentration of *E. coli* significantly (p<0.01). Shaheed (2021) found that lemongrass leaf powder and aqueous extract treatments significantly (p≤ 0.05) lowered the total bacterial count and *E. coli* count in the duodenum. El-Sahn et al. (2024) also showed a very significant decrease (p<0.01) in caecum *E. coli* count in lemongrass oil-treated groups compared to the control groups (Table 5).

Table 5: Effect of lemongrass essential oil on the gut microbial assay of broiler chickens

Gut microbial assay	Treatment groups				**S/*S/ N.S
	T_1	T_2	T_3	T_4	
E. coli count (log 10 cfu ml^{-1})	5.94^a	5.52^b	5.44^b	5.05^c	**S
Total viable count (log 10 cfu ml^{-1})	8.32^a	8.13^b	8.06^c	8.01^c	**S

Means with different superscripts in a row differ significantly *S: Significant at 5% probability (p<0.05); **S: Significant at 1% probability (p<0.01); NS: Non-significant (p>0.05)

Lemongrass essential oil had antibacterial properties due to its primary component, citral. Citral a mixture of geranial and neral (Mukhtar et al., 2013) and constituted more than 70% of lemongrass essential oil (Vazirian et al., 2012). Geraniol and nerol both had antibacterial activity against Gram-negative and Gram-positive pathogens. Citral interacted with the cytoplasmic membrane, causing membrane integrity loss, inhibition of respiratory enzymes, and eventually proton-motive force elimination, ultimately resulting in the death of the bacteria (Hasan-Al-Sharif et al., 2023).

4. CONCLUSION

Lemongrass essential oil (*Cymbopogon citratus*) improved nutrient metabolism, leading to higher feed utilization. It also reduced the gut microbe population, increasing nutritional bioavailability. However, no significant differences were observed in carcass characteristics. The results were favorable, but for lemongrass essential oil to be a viable alternative to antibiotic growth promoters, further research into other components, concentrations, and inclusion techniques is needed.

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