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Effect of cinnamon and peppermint essential oils on blood biochemistry, immunity, gut microbiota, and carcass traits in Japanese quail

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ABSTRACT

The present study was conducted to evaluate the effect of dietary supplementation essential oils of cinnamon and peppermint on blood biochemical parameters, immune response, gut microbiota, and carcass characteristics in Japanese quails. A total of 200 day-old Japanese quail chicks were randomly allotted to five treatment groups: T1 (basal/control diet), T2 (basal diet + cinnamon essential oil @ 500 ppm), T3 (basal diet + peppermint essential oil @ 500 ppm), T4 (basal diet + cinnamon + peppermint essential oils @ 250 ppm each), and T5 (basal diet + cinnamon + peppermint essential oils @ 500 ppm each) for a period of 35 days. Blood biochemical parameters remained within the normal physiological range in all treatment groups. Blood cholesterol concentration was reduced in T4, while creatinine levels were lower in all essential oil-supplemented groups as compared to the control. Liver enzyme activities did not differ significantly among treatments. Serum TIG concentration was significantly higher ($P<0.05$) in T3 and T4, whereas IgG concentration and lymphocyte count were significantly increased ($P<0.05$) in T2 and T4 as compared to the control. The heterophil/lymphocyte ratio was significantly higher ($P<0.05$) in T5. Caecal *Escherichia coli* counts were significantly reduced ($P<0.05$) in essential oil-supplemented groups. Dressing percentage improved significantly ($P<0.05$) in T2 and T3 with other carcass traits also showing improvement following essential oil supplementation. Overall, supplementation of cinnamon and peppermint essential oils, either individually or in combination, improved immune response, gut microbial balance, and carcass characteristics without exerting adverse effects on the health status of Japanese quails, indicating their potential as natural feed additives in quail production.

Keywords: Blood bio-chemicals, Carcass traits, Essential oils, Gut microbiota, Immune response, Japanese quail

The poultry sector is a rapidly growing sector, contributing significantly to food security and rural livelihoods. According to Basic Animal Husbandry Statistics (2025), India's poultry population has reached approximately 851.81 million birds. Globally, the poultry industry provides affordable, high-quality animal protein, and supports economic development and public health (Onogwu *et al.* 2024; Gabriel *et al.* 2025). Japanese quail (*Coturnix coturnix* japonica) has gained attention due to its rapid growth, early maturity, and high production efficiency. Its meat is valued for high protein content and superior nutritional quality (Luknov *et al.* 2023), with physicochemical properties comparable to chicken meat (Marareni and Mnisi, 2020). Age at slaughter significantly affects meat quality in Japanese quails, with older birds showing higher fat deposition and reduced tenderness, leading to lower consumer acceptability (Kokoszyński *et al.* 2024). Antibiotic growth promoters have long been used to improve poultry performance; however, their extensive use has raised concerns about antimicrobial resistance (Rahman *et al.* 2022, Mak *et al.* 2022; Wickramasuriya *et al.* 2024). Concerns over antibiotic residues in animal products have led to global restrictions, with the World Health Organization advocating reduced use and safer alternatives (WHO, 2023). Consequently, phytogenic compounds and essential oils have gained attention as natural feed additives due to their antimicrobial, antioxidant, and digestive-stimulatory properties, enhancing gut health, immunity, and nutrient utilization in poultry (Rehman *et*

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al. 2025). Essential oil of peppermint (*Mentha piperita*) contains menthol and polyphenolic compounds with strong antimicrobial and antioxidant activities (Hudz *et al.* 2023). Similarly, essential oil of cinnamon (*Cinnamomum spp.*) rich in cinnamaldehyde, exhibits potent antibacterial and digestive stimulant properties (Ali *et al.* 2021). Despite these benefits, information on the effects of essential oils in Japanese quails under Indian production conditions remains limited. Therefore, the present study evaluated the effect of dietary supplementation of essential oils cinnamon and peppermint on immune response, blood biochemical parameters, gut microflora, and carcass traits of Japanese quail.

MATERIALS AND METHODS

The study was conducted on 200 Japanese quail at the College of Veterinary and Animal Sciences, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, Uttar Pradesh, India, during March–April 2024. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC; Reg. No. 2058/GO/Re/SL/19/CPCSEA).

The experiment was conducted for 35 days a completely randomized design. A total of 200 day-old Japanese quail chicks were randomly allotted to five treatment groups with four replicates of 10 birds each (n=40 per treatment). Birds were reared under standard management and hygienic conditions on deep litter. During the first week, chicks were fed a standard broiler starter diet, after which they were individually weighed and randomly allocated to treatment groups based on uniform body weight. The dietary treatments were:

- T_1: Basal diet (control)
- T_2: Basal diet + cinnamon essential oil @ 500 ppm
- T_3: Basal diet + peppermint essential oil @ 500 ppm
- T_4: Basal diet + cinnamon essential oil @ 250 ppm + peppermint essential oil @ 250 ppm
- T_5: Basal diet + cinnamon essential oil @ 500 ppm + peppermint essential oil @ 500 ppm

Housing and management: The chicks were housed in a cage with similar housing and management conditions for the different treatment groups. Fresh and clean water was provided *ad lib*. Throughout the experimental period, the chicks were provided artificial light by electric bulbs of sufficient intensity.

Feed and feeding schedule: Standard broiler starter (0–3 weeks) and finisher (4–5 weeks) diets, formulated as per Bureau of Indian Standards (1992), was procured from Avian Nutrition and Feed Technology Division, Central Avian Research Institute, Izatnagar, Uttar Pradesh. Essential oils of cinnamon and peppermint sourced from Aromaaz International Ltd were incorporated into the basal diet at designated levels for each treatment. Feed was offered daily at 8:00 AM. Representative samples of essential oils and experimental diets were collected and analyzed for nutrient composition.

Nutrient analysis: Proximate composition of feed samples, including dry matter, crude protein, ether extract, crude fibre, and total ash, was determined following standard procedures of the Association of Official Analytical Chemists (AOAC 2005). Nitrogen was estimated by the Kjeldahl method, and crude protein was calculated as N × 6.25. Neutral detergent fibre and acid detergent fibre were determined using the Van Soest method.

Chemical composition of the experimental diet: The analyzed nutrient composition of the experimental basal diets (Table 1) confirmed that both starter and finisher diets were well-balanced and met the nutritional requirements of Japanese quail. Levels of crude protein, energy, fibre fractions, and essential minerals were within recommended standards for optimal growth and physiological performance (Lima *et al.* 2023; Carvalho *et al.* 2022; Khanipour *et al.* 2024).

Blood sampling and haematology: At the end of the experiment, blood samples were collected from one bird per replicate. Samples were taken in heparinized vials for hematological analysis, while the remaining blood was centrifuged at 3000 rpm for 10 min to obtain plasma. Plasma was used for biochemical, protein, metabolite, and enzyme analyses; glucose and enzyme assays were performed within 24 h, and the remaining samples were refrigerated until further parametrs analysis. Haemoglobin concentration was determined using Sahli's haemometer method, and packed cell volume (PCV) was measured by the Wintrobe method. Erythrocytic indices (MCV, MCH, and MCHC) were calculated using standard formulae. Total leukocyte count was estimated using a Neubauer haemocytometer with Turk's solution, while differential leukocyte count was determined from Leishman-stained blood smears under oil immersion. Absolute leukocyte counts were calculated based on differential percentages and total leukocyte count.

Blood biochemical parameters: Plasma glucose was estimated by the GOD–POD method (Kaplan, 1984). Serum creatinine was determined by the modified Jaffe's

Table 1. Analyzed nutrient contents of experimental diet (g/kg as DM)

Attributes	Experimental basal diet	
Chemical composition	Starter feed	Finisher feed
DM	876.5	854.5
ASH	149.8	152.6
CP	240.6	202.3
EE	41.2	54.2
CF	39.3	43.9
NFE	530.3	545.9
NDF	302.0	350.3
ADF	110.7	118.3
Ca	14	12.2
P	8.7	8.1

method using Erba kits, and cholesterol by the enzymatic method (Herbert, 1984) using Span Diagnostics kits. Total plasma protein and albumin were estimated by the Biuret and bromocresol green methods, respectively (Koller, 1984), while blood urea nitrogen (BUN) was measured by the enzymatic urease method (Newman and Price, 1997).

Liver enzymes, including ALT and AST, were analyzed using Erba kits following Murray (1994), and alkaline phosphatase (ALP) was estimated using Span Diagnostics kits based on p-nitrophenyl phosphate hydrolysis. Absorbance for respective parameters was recorded at standard wavelengths.

Immunoglobulin in blood: Total plasma immunoglobulin was estimated by zinc turbidity method described by McEwan *et al*. (1970). Plasma was allowed react with $ZnSO_4$ reagent, and turbidity was measured at 460 nm to calculate immunoglobulin concentration (mg/mL) using a standard curve. Plasma immunoglobulin G (IgG) was measured using a sandwich ELISA kit. After TMB substrate development, absorbance at 450 nm was read, and IgG concentration was determined from the standard curve.

Carcass traits and gut health assessment: At the end of the feeding trial, four birds from each treatment were randomly selected, fasted for 12 h, and weighed before slaughter. Birds were slaughtered ethically. After defeathering and evisceration, head, neck, and shanks were removed, and the dressed carcass weight was recorded. Cut-up parts (drumstick, thigh, wing, neck, back, and breast) and visceral organs (liver, heart, gizzard, and spleen) were weighed. The length and weight of the small intestine, large intestine, and caeca were also measured. Dressing percentage, ready-to-cook yield, and organ yields were calculated relative to post-fasting body weight.

Gut microbiota analysis: Ceacal contents (1 g) were collected aseptically in sterile tubes containing physiological saline and glycerol (9:1) and kept on ice. Samples were serially diluted and plated on selective media for enumeration of Escherichia coli. Colonies were identified on MacConkey lactose agar and confirmed on eosin methylene blue agar after incubation at 37 °C for 24 h. Results were expressed as log CFU/g of ceacal content.

Statistical analysis: Data were analyzed using a completely randomized design (CRD) through analysis of variance (ANOVA) as described by Snedecor and Cochran (1994) using SPSS software. Treatment means were compared using Duncan's Multiple Range Test (Duncan, 1955), and differences were considered significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

Hematological parameters and differential leukocyte count: The hematological profile and differential leukocyte count of Japanese quail birds at 5 weeks of age are presented in Tables 2 and 3. Dietary supplementation of essential oils significantly influenced hematological parameters, including hemoglobin, packed cell volume, erythrocyte count, leukocyte count, and erythrocytic indices (P<0.05),

Table 2. Hematological values of quails at 5 weeks of age fed with different levels of essential oil
Means bearing different superscripts within a column differ significantly at (P < 0.05)

Treatment	Hb (g/dL)	PCV (%)	TEC (%)	TEC (10^6/µl)	TLC (10^3/µL)	MCV (fL)	MCH (pg)	MCHG (%)
T_1	11.40^{a}	33.10^{a}	282.00^{a}	2.82^{a}	20.91^{a}	117.57^{a}	40.44	34.44
T_2	11.27^{ab}	34.87^{b}	316.00^{ab}	3.16^{ab}	23.55^{ab}	110.41^{ab}	35.70	32.30
T_3	13.04^{b}	35.11^{bc}	349.75^{bc}	3.50^{bc}	26.28^{b}	100.97^{b}	37.59	37.14
T_4	12.87^{a}	36.15^{c}	362.00^{c}	3.62^{c}	26.04^{b}	100.14^{b}	35.58	35.63
T_5	12.29^{a}	33.59^{a}	327.00^{bc}	3.27^{bc}	25.13^{b}	102.83^{b}	37.58	36.61
SEM	0.38	0.26	9.96	0.10	0.76	2.92	1.43	1.11
P value	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.16	0.05

Table 3. Hematological values of quails at 5 weeks of age fed with different levels of essential oil

Treatment	Differential leukocyte count (%)				
	Heterophils	Basophils + eosinophils	Lymphocytes	Monocytes	Heterophils/lymphocytes
T_1	23.66^{a}	5.23	69.53^{a}	2.20	0.34^{a}
T_2	23.85^{a}	5.04	69.47^{a}	3.01	0.35^{a}
T_3	22.75^{ab}	4.92	70.38^{ab}	2.46	0.32^{ab}
T_4	24.49^{c}	5.30	71.24^{b}	2.97	0.34^{a}
T_5	21.49^{bc}	5.59	70.74^{ab}	2.88	0.30^{b}
SEM	0.38	0.26	0.35	0.20	0.01
P VALUE	<0.01	0.46	<0.01	0.05	<0.01

Means bearing different superscripts within the same column differ significantly (P<0.05).

except mean corpuscular hemoglobin, which remained unaffected (P>0.05). These improvements indicated enhanced erythropoiesis, better oxygen-carrying capacity, and improved physiological status of the birds. The increase in total leukocyte count further reflected stimulation of the immune system. Significant variations in mean corpuscular volume and mean corpuscular hemoglobin concentration (P<0.05) suggested improved red blood cell integrity and functionality. These effects may be attributed to the bioactive compounds present in cinnamon (cinnamaldehyde) and peppermint (menthol), which possess antioxidant and immunomodulatory properties that enhance nutrient utilization and protect blood cells from oxidative damage. The differential leukocyte profile also showed significant modulation, with heterophils, lymphocytes, and the heterophil-to-lymphocyte (H/L) ratio being affected (P<0.01), while basophils and eosinophils remained unchanged (P>0.05), and monocytes showed marginal variation (P=0.05). An increase in lymphocyte proportion along with a reduced H/L ratio indicated improved immune competence and reduced physiological stress, as the H/L ratio is a well-established indicator of stress in avian species. The immunomodulatory effects of essential oils were likely due to their antimicrobial, anti-inflammatory, and antioxidant activities, which enhance immune cell function and reduce oxidative stress. These findings were consistent with previous reports and studies demonstrating that phytogenic feed additives significantly improve hematological traits, immune response, and stress resilience in poultry (Ghorbani *et al.*2025; Alagawany *et al.* 2021). Overall, dietary inclusion of essential oils significantly improved hematological health, immune status, and stress tolerance in Japanese quail birds.

Blood biochemical parameters: The blood biochemical profile of Japanese quail at 5 weeks of age is presented in Table 4. Dietary supplementation of essential oils significantly influenced several biochemical parameters, including creatinine, total protein, cholesterol, and albumin (P<0.05 to P<0.01), while glucose and urea remained unaffected (P>0.05), indicating that basic energy metabolism and nitrogen excretion were not altered by the treatments. The significant reduction (P<0.01) in serum creatinine in essential oil supplemented groups suggested improved kidney function and metabolic efficiency. Similarly, the significant increase (P<0.01) in total protein and albumin levels indicated enhanced protein synthesis and better nutritional status, reflecting improved liver function and overall physiological condition of the birds. A significant decrease (P<0.05) in serum cholesterol observed in supplemented groups demonstrated the hypolipidemic effect of essential oils, which might have contributed to improved lipid metabolism and cardiovascular health. These effects are likely to be associated with the bioactive compounds present in cinnamon (cinnamaldehyde) and peppermint (menthol), known for their antioxidant, hepatoprotective, and lipid-lowering properties. The absence of significant changes (P>0.05) in glucose and urea levels suggested that essential oil supplementation did not adversely affect carbohydrate metabolism or protein catabolism, thereby maintaining metabolic homeostasis. The observed improvements in protein profile and lipid metabolism are in agreement with earlier findings (Abbas *et al.* 2025), who reported enhanced biochemical parameters and reduced cholesterol levels in quails and poultry fed phytogenic additives. Recent studies further support that essential oils significantly improved the biochemical indices by reducing oxidative stress and enhancing liver function in poultry (Khanipour *et al.* 2024). Basil and peppermint essential oils supplementation (250-500 mg/kg) in quail diets has been reported earlier to increase total protein, globulin, and albumin, while reducing total cholesterol, with breed-specific effects **(**Abbas *et al.* 2021**).** Overall, dietary inclusion of essential oils positively modulated blood biochemical parameters, indicating improved metabolic efficiency, liver function, and health status in Japanese quail.

Liver enzyme: Liver enzyme activities of Japanese quail at 5 weeks of age (Figure 1) were not significantly affected (P>0.05) by dietary supplementation of essential oils, as indicated by unchanged serum AST, ALT, and ALP levels. This suggested that cinnamon and peppermint essential oils did not impair liver function or induce hepatotoxicity. The stable enzyme profile reflected a normal hepatic metabolism and good physiological adaptation of birds to the treatments. These findings agreed with previous studies (Abbas *et al.* 2025; Khanipour *et al.* 2025), confirming that phytogenic feed additives are safe and do not adversely affect liver health in poultry.

Table 4. Blood biochemical parameters of quails at 5th week of age fed with different levels of essential oil

Treatment	Glucose (mg/dl)	Creatinine (mg/dl)	Cholesterol (mg/dl)	Total protein (g/dl)	Urea (mg/dl)	Albumin g/dl
T_1	225.72	0.63^a	202.83^a	3.60^a	30.86	1.30^a
T_2	222.41	0.60^b	192.99^{ab}	4.25^b	30.82	1.41^{ab}
T_3	221.26	0.58^{ab}	194.88^{ab}	4.35^b	26.68	1.38^{ab}
T_4	221.12	0.56^c	188.41^b	4.62^b	30.91	1.46^b
T_5	224.29	0.58^c	193.53^{ab}	4.10^{ab}	28.63	1.34^{ab}
SEM	1.64	0.01	2.47	0.14	1.58	0.03
P value	0.26	<0.01	0.01	<0.01	0.27	0.01

Means bearing different superscript in a column differ significantly (P<0.05)

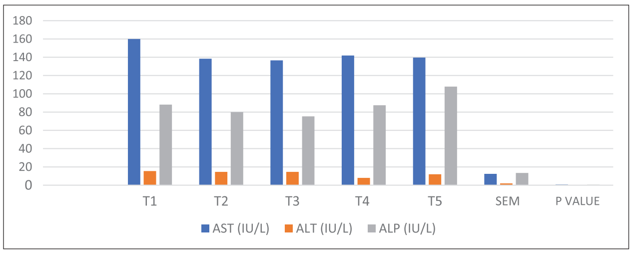

Fig. 1. Blood biochemical parameters (Liver Enzyme) of quail birds at 5 weeks of age fed with different levels of essential oil

Immunocompetence traits: The immune response of Japanese quail at 5 weeks of age (Figure 2) showed that dietary essential oils had no significant effect (P>0.05) on total immunoglobulin levels, although a numerical increase was observed. However, IgG levels were significantly improved (P<0.01), indicating enhanced humoral immunity and better disease resistance in supplemented groups. This improvement may be attributed to bioactive compounds such as cinnamaldehyde and menthol, which possess antioxidant and immunostimulatory properties, promoting antibody production and reducing oxidative stress. The unchanged total immunoglobulin alongside increased IgG suggested selective enhancement of specific immune components. These findings aligned with earlier studies (Ali *et al.* 2021; Ranwa *et al.* 2023; Khanipour *et al.* 2025), confirming that essential oils improve immune response and immunocompetence in poultry without adverse effects.

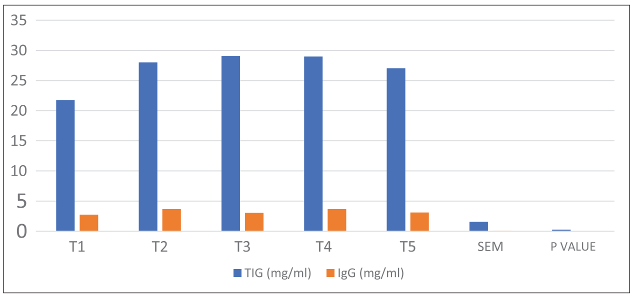

Fig. 2. Total immunoglobin and immunoglobin G (IgG) response of quail birds at 5th week of age fed with different levels of essential oil

Gut microbiota: The gut microbial population of Japanese quail at 35 days of age (Figure 3) showed that dietary supplementation of essential oils significantly reduced (P<0.01) ceacal *Escherichia coli* counts compared to the control. This indicated improved intestinal microbial balance and gut health in supplemented birds. The antimicrobial effect is likely to be due to bioactive compounds such as cinnamaldehyde and menthol, which inhibit harmful bacteria by disrupting cell membranes and enzyme activity. Reduced *E. coli* levels also suggest better gut integrity and nutrient utilization. The findings aligned with previous studies (Abbas *et al.* 2021; Khanipour *et al.* 2024), confirming that phytogenic additives help suppress pathogenic microbes and promote a healthier intestinal

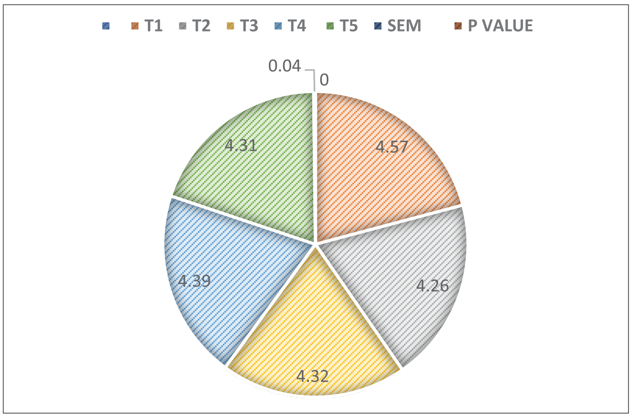

Fig. 3. Gut E. coli Analysis of Intestinal Tract (Ceacum Contents) at 5 Weeks of Age Fed with Different Levels of Essential Oil in Japanese Quails

environment, although responses may vary with dosage and dietary factors.

Carcass characteristics: Carcass characteristics of Japanese quail at 35 days of age (Figure 4) showed significant effects of dietary essential oils. Shrinkage percentage (4.61–5.68%) was significantly lower (P<0.01) in T_4, while dressing percentage (73.41–78.83%) was significantly higher (P<0.01) in T_2 and T_3 groups. Eviscerated weight (54.12–61.71%) and ready-to-cook yield (58.47–66.66%) also varied significantly (P<0.05). Liver weight was significantly affected (P<0.01), whereas heart and gizzard weights remained unchanged (P>0.05). The improved dressing percentage and carcass yield in supplemented groups may be due to better nutrient utilization and metabolic efficiency from phytogenic compounds in cinnamon and peppermint oils. These findings are supported by previous studies (Deminic *et al.* 2022; Abbas *et al.* 2024; Chigede *et al.* 2025), although responses may vary depending on the type and inclusion level of essential oils.

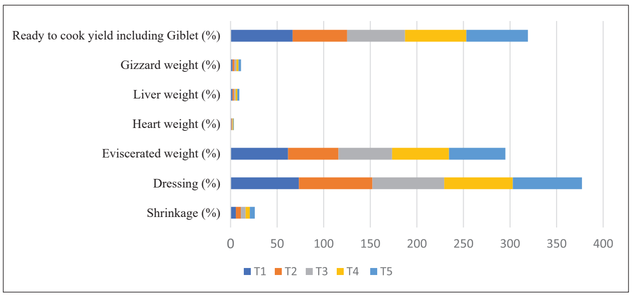

Fig. 4. Carcass quality characteristics at 5 weeks of age of quail fed with the effect of feeding different levels of essential oils

Slaughter traits: The cut-up parts of Japanese quail at 5 weeks of age (Figure 5) showed significant variation with dietary essential oil supplementation. Thigh (11.77–14.13%), drumstick (8.46–10.22%), breast (44.59–46.64%), and neck (3.35–3.75%) percentages were

Table 5. Development of digestive organ and carcass quality characteristics of japanese quails at 5 weeks of age fed with different level of essential oil Means bearing different superscript in a column differ significantly (P<0.05)

Treatment	Proventriculus (%)	Small intestine weight (%)	Small intestine Cm (%)	Large intestine wt (%)	Large intestine (cm/100gm) (%)	Ceacal weight (%)	Ceacal length (cm/100gm)	Spleen weight (%)
T_1	1.89^{a}	5.10^{a}	8.55^{ab}	0.14	0.50^{a}	0.74^{a}	0.87^{a}	0.14
T_2	1.60^{ab}	3.70^{bc}	8.33^{a}	0.14	0.63^{b}	0.66^{a}	0.83^{a}	0.14
T_3	1.51^{b}	5.56^{c}	9.62^{d}	0.17	0.60^{b}	0.70^{a}	0.65^{b}	0.11
T_4	1.70^{ab}	4.74^{b}	8.89^{bc}	0.12	0.61^{b}	0.53^{b}	0.73^{c}	0.11
T_5	1.54^{ab}	5.19^{a}	9.32^{cd}	0.13	0.59^{b}	0.67^{a}	0.69^{bc}	0.12
SEM	0.08	0.13	0.11	0.01	0.02	0.03	0.02	0.01
P VALUE	0.03	<0.01	<0.01	0.13	<0.01	<0.01	<0.01	0.11

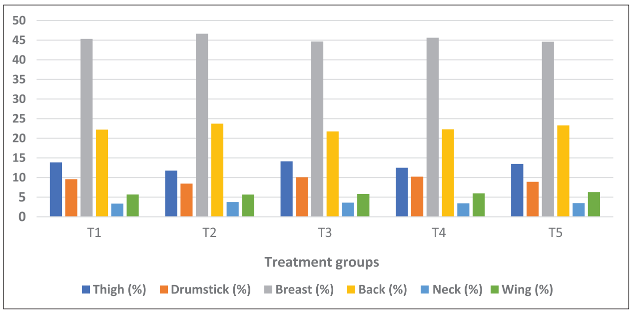

Fig .5. Cut-up parts carcass quality characteristics at 5 weeks of age of quail fed with the effect of feeding at different levels of essential oils

significantly affected (P<0.05 to P<0.01), with higher values observed mainly in T_2, T_3, and T_4 groups. However, back and wing percentages remained unaffected (P>0.05). The improvement in breast and drumstick yield may be attributed to enhanced nutrient utilization and growth-promoting effects of phytogenic compounds in cinnamon and peppermint oils. These findings were consistent with earlier studies (Abbas *et al.* 2021; Deminic *et al.* 2022; Abbas *et al.* 2025), although some variation may be expected depending on the type and inclusion level of essential oils.

Development of carcass quality characteristics: The development of digestive organs in Japanese quail at 35 days (Table 5) showed significant effects of dietary treatments. Proventriculus weight differed (P<0.05), while small intestine weight and length, large intestine length, and ceacal traits were significantly influenced (P<0.01), with higher values observed in essential oil-supplemented groups, particularly T_3. However, large intestine weight and spleen weight remained unaffected (P>0.05). The increased intestinal size indicated enhanced gut development and digestive capacity, which is likely to improve nutrient absorption. These effects may be attributed to the antimicrobial and digestive stimulant properties of bioactive compounds in cinnamon and peppermint oils. Similar findings were reported by Abbas *et al*. (2021) and more recently by Khan *et al*. (2023), who observed improved gut morphology and nutrient utilization in poultry supplemented with phytogenic feed additives.

Dietary supplementation of cinnamon oil (500 ppm), peppermint oil (500 ppm), and their combination (250 ppm each) improved hematological profile, immune response, gut microbiota, and carcass traits in Japanese quail. The combination supplementation showed better overall performance without any adverse effect on blood biochemical parameters or liver enzymes, indicating that these essential oils can serve as safe and effective phytogenic feed additives to enhance quail health and productivity.

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