International Journal of Agricultural Technology 2026 Vol. 22(1):55-70
DOI: https://doi.org/10.63369/ijat.2026.22.1.55-70
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Effects of thymol–carvacrol combined with organic acids on carcass traits, meat quality, and stress indicators in broilers under enteric disease challenge

Busayakanit, P.[1], Srikijkasemwat, K.[1*], Philatha, A.[2], Rassmidatta, K.[2], Ruangpanit, Y.[2], Yan, F.[3], Romero-Sanchez, H.[3], Siwapirunthep, P.[4] and Chanporn, C.[4]

[1]Department of Animal Production Technology and Fisheries, School of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok 10520, Thailand; [2]Department of Animal Sciences, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom 73140, Thailand; [3]Novus International, Inc. 17988 Edison Ave, Chesterfield, Missouri 63005, United States; [4]Department of Agricultural Education, School of Industrial Education and Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok 10520, Thailand.

Busayakanit, P., Srikijkasemwat, K., Philatha, A., Rassmidatta, K., Ruangpanit, Y., Yan, F., Romero-Sanchez, H, Siwapirunthep, P. and Chanporn, C. (2026). Effects of thymol–carvacrol combined with organic acids on carcass traits, meat quality, and stress indicators in broilers under enteric disease challenge. International Journal of Agricultural Technology 22(1):55-70.

Abstract The effects of a feeding program combining thymol–carvacrol and organic acids were evaluated as an alternative to an antibiotic growth promoter (AGP) in broilers under a coccidial challenge. A total of 750 male Ross 308 chicks were assigned to a negative control (T1), an AGP group with bacitracin methylene disalicylate (T2), or a thymol-carvacrol–organic acid group (T3). Slaughter weight, dressing percentage, retail cut yields, and meat quality traits did not differ among treatments ($P > 0.05$). T2 had the highest number of birds without foot pad lesions, significantly exceeding T1 and comparable to T3, which also outperformed T1 ($P < 0.05$). T2 recorded the fewest mild and severe lesions, while T3 showed a numerical reduction in severe lesions compared with T1. Both T2 and T3 reduced lesion severity relative to T1, with T2 showing the most favorable scores and T3 demonstrating meaningful potential as a non-antibiotic alternative. Thigh meat malondialdehyde (MDA) levels increased during storage ($P < 0.01$), but no treatment effects were observed ($P > 0.05$). Serum MDA and the proportions of lymphocytes, monocytes, eosinophils, and basophils were unaffected; however, heterophil percentage and the heterophil-to-lymphocyte ratio were significantly higher in T1 than in T2 and T3 ($P < 0.01$), indicating reduced physiological stress with both AGP and thymol-carvacrol–organic acid supplementation. Overall, thymol–carvacrol with organic acids-maintained carcass yield and meat quality while mitigating stress responses and improving foot pad health, supporting its potential as a viable alternative to AGPs in broilers under enteric challenge.

Keywords: Phytogenic feed additive, Carcass composition, Meat quality, Lipid oxidation, White blood cell profiling

[*] **Corresponding Author**: Srikijkasemwat, K.; **Email:** kanokrat.sr@kmitl.ac.th

Introduction

The broiler industry is one of the most rapidly growing sectors in global animal agriculture, driven by rising demand for affordable, high-quality animal protein. Chicken meat is particularly valued by consumers due to its favorable nutritional profile, including low fat content, high digestibility, and excellent protein quality (Kaewhom, 2018; Voitsekhivska *et al*., 2021). To meet these growing demands sustainably, improving growth performance and meat quality in broiler production is essential (Nakavisut *et al*., 2019; Srikijkasemwat *et al*., 2025).

For decades, antibiotic growth promoters (AGPs) have been routinely included in poultry diets to enhance productivity and prevent enteric diseases. However, the long-term use of antibiotics in animal feed has raised serious public health concerns, notably the development of antibiotic-resistant bacteria and the presence of drug residues in meat products (Somkuna and Somkuna, 2018). These issues have led to increasing regulatory restrictions and a growing interest in natural, safe alternatives to in-feed antibiotics.

Phytogenic feed additives—bioactive compounds derived from herbs, spices, and other plants—have emerged as promising candidates to replace AGPs. These compounds are known for their antimicrobial, antioxidant, anti-inflammatory, and immunomodulatory properties (Ajin and Thapingkae, 2014; Li *et al*., 2012). Among the most widely studied phytogenics are thymol and carvacrol, two phenolic constituents found abundantly in essential oils extracted from plants of the Lamiaceae family, such as *Thymus vulgaris* (thyme) and *Origanum vulgare* (oregano) (Windisch *et al*., 2008).

Thymol–carvacrol exert strong antimicrobial effects by disrupting bacterial cell membranes, leading to leakage of cellular contents, pH imbalance, and inhibition of essential metabolic processes (Burt, 2004; Ultee *et al*., 2002). These compounds also interfere with bacterial quorum sensing, which plays a critical role in virulence regulation and biofilm formation (Kerekes *et al*., 2013). In poultry, thymol–carvacrol have been shown to reduce pathogenic bacteria such as *Escherichia coli*, *Salmonella* spp., and *Clostridium perfringens*, while promoting beneficial microbiota including *Lactobacillus* spp. and *Bifidobacterium* spp., thereby enhancing gut health and nutrient absorption (Hashemipour *et al*., 2013; Giannenas *et al*., 2014).

In addition to their antimicrobial activity, thymol–carvacrol possess potent antioxidant properties. They act as free radical scavengers, interrupting lipid peroxidation chain reactions and reducing malondialdehyde (MDA) formation—a key marker of oxidative spoilage in meat (Brenes and Roura, 2010; Saleh *et al*., 2019). By limiting oxidative damage, these compounds may help preserve meat quality during storage by improving color stability, tenderness, and shelf life.

Phytogenic feed additives, beyond their antimicrobial and antioxidant effects, may also modulate stress and immune responses in broilers. Hematological parameters—including heterophil, lymphocyte, monocyte, eosinophil, and basophil counts, and the heterophil-to-lymphocyte (H:L) ratio—are established indicators of stress and health in poultry (Muneer *et al*., 2021; Nwaigwe *et al*., 2020). Under enteric disease challenge, these indices, along with serum MDA levels, were evaluated to assess the potential of thymol–carvacrol combined with organic acids to reduce stress and support productivity (Brenes and Roura, 2010; Saleh *et al*., 2019). Despite promising findings, results across studies remain inconsistent due to variations in dosage, delivery methods, animal health status, and environmental conditions. Further research is therefore warranted to evaluate the practical efficacy of thymol–carvacrol, particularly under commercial-like conditions and disease challenges.

The present study aimed to investigate the effects of dietary supplementation with thymol–carvacrol, combined with organic acids, on carcass characteristics, thigh meat quality, lipid oxidation, and white blood cell profiling in broiler chickens subjected to an enteric challenge with a high-dose coccidiosis vaccine.

Materials and methods

Animal ethics

This study was approved by and carried out in compliance with ethical standards from the Animal Care and Use Committee of Kasetsart University, Kamphaeng Saen Campus, Thailand (Approval number: ACKU68-AGK-010).

Animals, diets, treatments, and raising procedures

A total of 750 one-day-old male Ross 308 broiler chicks were used and reared for 42 days at the Animal Science Research and Development Center, Faculty of Agriculture, Kasetsart University, Kamphaeng Saen Campus, Thailand. Chicks were randomly assigned to three dietary treatments with 10 replicates per treatment and 25 birds per replicate. T1 is the negative control, received a basal diet without any supplementation, T2 is the positive control, received the basal diet with bacitracin methylene disalicylate (BMD) at 50 g/ton or antibiotic group, and T3 received thymol-carvacrol at 30 g/ton without organic acid during days 1–28 and a combination of thymol-carvacrol (15 g/ton) and organic acid (250 g/ton) during days 29–42. Thymol-carvacrol (NE150®, NEXT

ENHANCE®, Novus International Inc., USA) is a phytogenic feed additive containing thymol-carvacrol (1:1), while organic acid (Provenia®, Novus International) is a protected-release aromatic organic acid formulation containing benzoic acid, designed to gradually release active compounds along the gastrointestinal tract to enhance intestinal health and microbiota balance. The diets were formulated to meet or exceed NRC (1994) standards. Birds were housed in an evaporative-cooled facility with *ad libitum* access to feed and water. All birds were vaccinated against Newcastle disease (ND), infectious bronchitis (IB), and infectious bursal disease (IBD) were administered at 7, 14, and 21 days of age, and then stress challenged with a live coccidiosis vaccine (FORTEGRA®) containing *E. tenella*, *E. acervulina*, *E. mivati*, and *E. maxima* at 20x dosage on day 14 days of age. The composition of the basal diet is shown in Table 1.

Table 1. Ingredient compositions and calculated analysis of experimental basal diet

	Starter	**Grower**	**Finisher**
Ingredient (%)			
Corn 8%	53.96	56.40	60.48
Soybean meal (48% CP)	36.36	31.56	25.30
Full fat soybean	2.00	4.00	6.00
Rice bran oil	2.54	3.40	3.98
L-Lysine HCl	0.22	0.17	0.16
DL-Methionine	0.34	0.29	0.27
L-Threonine	0.14	0.09	0.06
Mono-dicalcium phosphate 21%	2.20	1.96	1.72
Limestone	1.20	1.09	0.99
Salt	0.29	0.31	0.31
Broiler vit/min premix	0.20	0.20	0.20
Choline Chloride 60%	0.10	0.09	0.09
Pellet binder	0.30	0.30	0.30
Sodium bicarbonate	0.15	0.13	0.13
Total	100	100	100
Calculated analysis			
Metabolizable Energy for poultry (kcal/kg)	3000.00	3100.00	3200.00
Crude protein (%)	23.00	21.50	19.50
Crude fat (%)	5.36	6.62	7.63
Crude fiber (%)	3.39	3.26	3.08
Ash (%)	6.37	5.90	5.37
Calcium (%)	0.96	0.87	0.78
Total phosphorus (%)	0.83	0.77	0.70
Available phosphorus (%)	0.48	0.44	0.39

Lysine (%)	1.44	1.30	1.16
Methionine (%)	0.69	0.62	0.57
Methionine + Cysteine (%)	1.08	0.99	0.91
Choline (mg/kg)	1700.00	1600.00	1500.00
Sodium (%)	0.16	0.16	0.16
Chloride (%)	0.23	0.23	0.23

[1] Premix contains minerals and vitamins per 1 kg feed, including Vitamin A 6,000,000 IU, Vitamin D3 1,200,000 IU, Vitamin E 30.00 g, Vitamin K 1.50 g, Vitamin B1 1.50 g, Vitamin B2 4.00 g, Vitamin B6 2.00 g, Vitamin B12 0.01 g, Niacin 25.00 g, Pantothenic acid 7.50 g, Biotin 0.20 g, Folic acid 1.00 g, Copper 7.50 g, Iron 20.00 g, Manganese 50.00 g, Zinc 50.00 g, Iodine 0.50 g, Selenium 0.15 g, Antioxidant 2.50 g, and Filler (rice hull ground only) 554.29 g.

Data collection

Carcass sampling and evaluation

At 42 days of age, one bird from each of the 10 replicates per treatment (10 birds per group) was chosen for carcass evaluation, totaling 30 birds. Parameters recorded included live body weight, defeathered weight, hot and cold carcass weights, dressing percentage, and retail cut yields (breast, thigh, drumstick, wing, back, shank and foot, head and neck, and abdominal fat).

Dressing percentage was calculated as the ratio of cold carcass weight to live body weight. Carcasses were processed following standard industrial procedures, including scalding, defeathering, evisceration, and immersion chilling. Chilling involved rinsing in clean water, followed by immersion in chilled water at 10 °C for 10 minutes and then at 4 °C for 15 minutes with agitation. Carcasses were drained prior to cutting.

Foot pad score

At trial end, foot pad dermatitis was scored in all birds using a three-grade scale (Aviagen, 2012): 0 = no or minor superficial lesions; 1 = mild lesions with discoloration or dark papillae; 2 = severe lesions with large ulcers, hemorrhage, or pronounced swelling.

Meat quality assessment

Following slaughter, left thigh samples were collected from each carcass sample and stored in zip-lock plastic bags at 10 °C prior to assessments. The following meat quality parameters were evaluated: pH at 3 and 24 hours postmortem using a portable pH meter (Seven2Go™, Mettler Toledo, Switzerland); color attributes including lightness (L*), redness (a*), and yellowness (b*) assessed using a (MiniScan EZ, Hunterlab, USA); water-holding capacity determined by measuring drip loss, purge loss, and cooking loss percentages; sarcomere length was measured using a helium-neon laser

(31004 model, REO, Boulder, Colorado, USA); muscle fiber diameter (µm) was measured using a compound microscope (CX23, Olympus, Japan) at 40× magnification.; and shear force (kgf/cm^2) evaluated using a texture analyzer (EZ-SX, Shimadzu, Japan) equipped with a Warner-Bratzler blade.

Lipid oxidation analysis

Lipid oxidation in thigh meat was assessed during refrigerated storage at 4 °C on days 1, 3, and 5 postmortems by determining malondialdehyde (MDA) levels using the thiobarbituric acid reactive substances (TBARs) assay, as described by Grotto *et al*. (2007). The absorbance of the reaction mixture was measured at 532 nm using a UV-visible spectrophotometer (Jenway 7315, Cole-Parmer, UK) to indicate oxidative stress.

White blood cell profiling and malondialdehyde analysis

One chicken was randomly selected from each replicate for blood collection. A volume of 3 mL of blood was drawn from the wing vein of each bird. The collected blood was divided into two portions. One portion was stored in EDTA tubes for white blood cell (WBC) analysis, and smears were prepared and stained using the Wright–Giemsa method. WBC—including heterophils (H), lymphocytes (L), monocytes, eosinophils, and basophils—were enumerated under 100× magnification using DP73 software, and stress status was evaluated by calculating the heterophil-to-lymphocyte (H:L) ratio as described by Gross and Siegel (1983).

The other portion of blood was centrifuged at 3,000 rpm at 25 °C for 5 minutes to separate serum, which was then stored at −20 °C for MDA analysis. MDA levels were measured using the thiobarbituric acid reactive substances (TBARS) assay, and absorbance at 532 nm was determined with a UV–visible spectrophotometer following the procedure of Grotto *et al.* (2007) as an indicator of oxidative stress.

Statistical analysis

The experiment was conducted using a completely randomized design (CRD) with three dietary treatments. The carcass and meat quality data were analyzed using one-way analysis of variance (ANOVA). When significant differences were detected ($P < 0.05$), Duncan's New Multiple Range Test (DMRT) was applied for mean separation. The statistical model used was

$$Y_{ij} = \mu + A_i + E_{ij}$$

Where Y_{ij} is the values of the dependent variable, μ is the overall meaning, A_i is the treatment effect, and E_{ij} is the random error.

Meat lipid oxidation determined by MDA on 1, 3, and 5 days after stored in refrigerator, data were analyzed using repeated measures designs which MDA from all treatments were measured at 3 different points of time. The model is as follows.

$$Y_{ij} = \mu + A_i + T_j + AT_{ij} + E_{ijk}$$

Where Y_{ij} is the values of the dependent variable, μ is the overall meaning, A_i is the treatment effect, T_j is time effect, AT_{ij} is the interaction between treatment and time, and E_{ij} is the random error.

Each foot pad score category (0, 1, and 2) was analyzed separately using the Kruskal–Wallis test, followed by Dunn's post-hoc test with Bonferroni adjustment when $p < 0.05$. Results are reported as mean ± SD with significance denoted by different letters.

All statistical analyses were performed using SAS (SAS Institute Inc., Cary, NC, USA).

Results

Carcass composition

The effects of dietary supplementation with thymol-carvacrol combined with organic acid on carcass characteristics and retail cut yields in broilers at 42 days of age are presented in Table 2. No statistically significant differences ($P > 0.05$) were observed among treatment groups in initial body weight, slaughter weight, or chilled dressing percentage. Initial body weight ranged from 43.34 to 43.39 g, slaughter weight from 3233.33 to 3295.62 g, and chilled dressing percentage from 85.73% to 86.31%. Similarly, yields of carcass components including breast, thigh, drumstick, wing, back, shank and foot, head and neck, and abdominal fat did not differ significantly among treatments. Breast yield ranged from 33.09% to 34.42%, thigh from 16.06% to 16.62%, and drumstick from 12.13% to 12.34%. Other components such as abdominal fat (1.15-1.37%) and head and neck (5.68-6.11%) also showed no statistical variation among groups. These results indicated that thymol–carvacrol supplementation combined with organic acids had no adverse effects on carcass yield or composition under the conditions tested.

Foot pad score

The antibiotic group (T2) had the highest number of birds without lesions (score 0; 19.3 ± 1.42), significantly greater than the control (T1; 17.1 ± 1.37) and comparable to the phytogenic group (T3; 18.0 ± 1.70) (Figure 1). The phytogenic group also showed a significantly higher number of birds without lesions compared with T1 ($P < 0.05$), indicating its potential to prevent lesion development. For mild lesions (score 1), T2 had the lowest count (2.2 ± 1.03), significantly lower than both T1 (3.9 ± 1.10) and T3 (3.1 ± 1.10), which did not differ from each other. For severe lesions (score 2), T2 again recorded the lowest value (0.3 ± 0.48), significantly lower than T1 (1.5 ± 0.85) and T3 (1.0 ± 0.47). Although there was no significant difference between T1 and T3 for severe lesions, T3 showed a numerically lower value than T1, suggesting a possible trend toward reducing lesion severity. Overall, both T2 and T3 reduced lesion severity compared with T1, with T2 showing the most favorable overall foot pad scores and T3 demonstrating meaningful potential as a non-antibiotic alternative.

Table 2. Effects of thymol–carvacrol supplementation combined with organic acids on carcass composition of broiler at 42 days of age

Item	**Treatments**			**SEM**	**P-value**
	T1	T2	T3		
Initial body weight (gram)	43.34	43.39	43.34	0.13	0.612
Slaughter weight (gram)	3233.33	3295.62	3277.00	29.77	0.482
Chill carcass weight (gram)	2772.33	2844.37	2808.00	26.36	0.303
Chill dressing percentage (%)	85.75	86.31	85.73	0.50	0.746
Retail cut (%)					
Breast	33.55	33.09	34.42	0.61	0.492
Thigh	16.20	16.62	16.06	0.27	0.462
Drumstick	12.13	12.34	12.16	0.16	0.696
Wing	8.61	8.51	8.58	0.10	0.829
Back	18.38	18.28	18.11	0.26	0.858
Shank and foot	3.77	3.67	3.60	0.09	0.674
Head and neck	5.81	6.11	5.68	0.14	0.210
Abdominal Fat	1.37	1.15	1.21	0.11	0.492

T1 (negative control standard diet without antibiotic) T2 (positive control add antibiotic – bacitracin methylene disalicylate 50 g/t all phases), and T3 (standard diet + thymol-carvacrol 30 g/ton starter and grower phase (0-28 d), organic acid 250 g/ton + thymol-carvacrol 15 g/ton finisher phase (29-42 d).

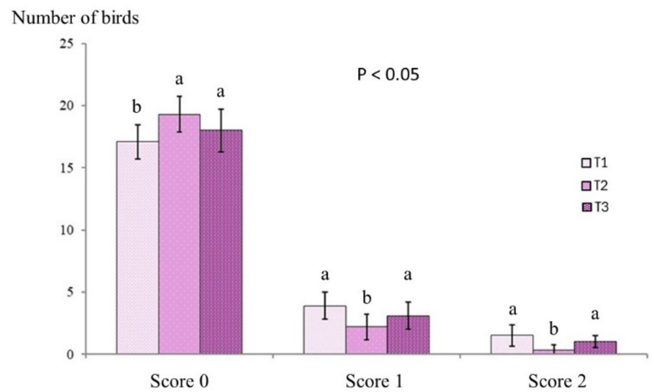

Figure 1. Effects of thymol–carvacrol supplementation combined with organic acids on foot pad score: T1 (negative control standard diet without antibiotic) T2 (positive control add antibiotic – bacitracin methylene disalicylate 50 g/t all phases), and T3 (standard diet + thymol-carvacrol 30 g/ton starter and grower phase (0-28 d), organic acid 250 g/ton + thymol-carvacrol 15 g/ton finisher phase (29-42 d)

Thigh meat quality

The effects of dietary treatments on thigh meat quality parameters is shown in Table 3. No significant differences (P > 0.05) were found among groups in pH measured at 3 and 24 hours postmortem, with values ranging from 6.32 to 6.48 and 6.25 to 6.41, respectively. Color attributes, including L*, a*, and b*, were also unaffected by treatment. L* values ranged from 60.27 to 62.25, a* from 3.11 to 3.67, and b* from 12.98 to 13.55.

Water-holding capacity, assessed via drip loss, purge loss, and cooking loss, showed no significant differences among treatments. Drip loss and purge loss were minimal and consistent across groups (0.94–0.95% and 0.63–1.03%, respectively), while cooking loss ranged from 14.49% to 16.45%. Texture-related traits such as sarcomere length, muscle fiber diameter, and shear force were also similar among treatments (P >0.05).

Malondialdehyde concentrates in thigh meat

The effects of treatment and storage duration on MDA concentrations in broiler thigh meat are shown in Figure 2. Storage time significantly affected MDA levels (P < 0.01), while treatment and the treatment × storage time interaction were not significant (P > 0.05). MDA concentrations increased progressively with storage duration (P < 0.01).

Table 3. Effects of thymol–carvacrol supplementation combined with organic acids on thigh meat quality of broiler at 42 days of age

Item	Treatments			SEM	P-value
	T1	T2	T3		
pH 3 h	6.48	6.32	6.36	0.030	0.076
pH 24 h	6.41	6.25	6.28	0.031	0.065
Lightness (L*)	62.25	61.90	60.27	0.448	0.157
Redness (a*)	3.11	3.67	3.42	0.151	0.329
Yellowness (b*)	12.98	13.17	13.55	0.245	0.642
% Drip loss	0.94	0.95	0.95	0.125	0.999
% Purge loss	0.93	1.03	0.63	0.085	0.126
% Cooking loss	14.49	16.45	14.96	0.525	0.293
Sarcomere length (µm)	3.34	3.21	3.23	0.032	0.230
Fibre diameter (µm)	66.33	66.33	61.56	1.278	0.218
Shear force (kgf/cm^2)	2.70	3.24	3.11	0.146	0.299

T1 (negative control standard diet without antibiotic) T2 (positive control add antibiotic – bacitracin methylene disalicylate 50 g/t all phases), and T3 (standard diet + thymol-carvacrol 30 g/ton starter and grower phase (0-28 d), organic acid 250 g/ton + thymol-carvacrol 15 g/ton finisher phase (29-42 d)

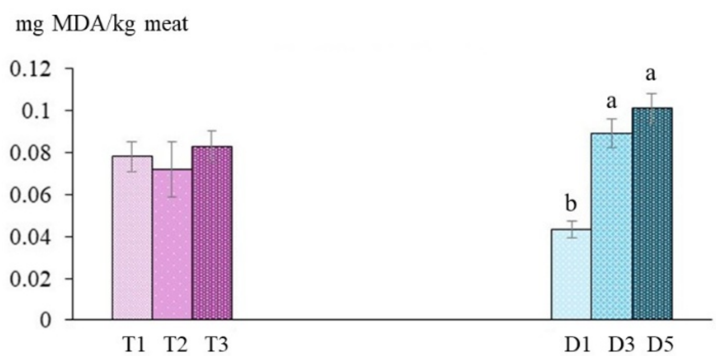

Figure 2. Effect of thymol–carvacrol supplementation combined with organic acids and storage time on thigh meat oxidation: T1 (negative control standard diet without antibiotic) T2 (positive control add antibiotic – bacitracin methylene disalicylate 50 g/t all phases), and T3 (standard diet + thymol-carvacrol 30 g/ton starter and grower phase (0-28 d), organic acid 250 g/ton + thymol-carvacrol 15 g/ton finisher phase (29-42 d). D = storage days (1, 3, and 5 days at 4 °C). [a, b] P <.001

Serum malondialdehyde levels and white blood cell profile

The effects of dietary supplementation with thymol-carvacrol combined with organic acid on serum malondialdehyde levels and white blood cell profile are presented in Table 4. No statistically significant differences (P > 0.05) were observed among treatment groups in serum malondialdehyde levels, lymphocyte, monocyte, eosinophil, or basophil percentage. While the percentage of heterophile and H:L ratio were higher in T1 than T2 and T3 (P < 0.01).

Table 4. Effect of thymol–carvacrol supplementation combined with organic acids on serum malondialdehyde levels and white blood cell profile of broilers at 28 days of age

Item	Treatments			SEM	P-value
	T1	T2	T3		
Malondialdehyde (nmol/mL)	8.97	9.01	9.05	0.127	0.625
Heterophil (%)	40.50 [a]	31.20 [b]	27.00 [b]	1.690	0.003
Lymphocyte (%)	42.33	45.00	47.25	2.750	0.680
Monocyte (%)	4.90	8.80	7.10	1.350	0.141
Eosinophil (%)	4.40	6.90	9.10	1.840	0.213
Basophil (%)	7.90	10.40	9.90	1.300	0.369
H/L ratio	0.97 [a]	0.71 [b]	0.58 [b]	0.050	0.008

T1 (negative control standard diet without antibiotic) T2 (positive control add antibiotic – bacitracin methylene disalicylate 50 g/t all phases), and T3 (standard diet + thymol-carvacrol 30 g/ton starter and grower phase (0-28 d), organic acid 250 g/ton + thymol-carvacrol 15 g/ton finisher phase (29-42 d). [a, b] P <.001

Discussion

This study evaluated the effects of dietary supplementation with thymol–carvacrol combined with organic acids on carcass traits, meat quality, lipid oxidation, and physiological stress in broilers under enteric challenge induced by a high-dose coccidiosis vaccine.

Carcass performance parameters, including slaughter weight, dressing percentage, and retail cut yields, were not significantly affected by dietary treatments. These findings align with Hashemipour *et al.* (2013) and Giannenas *et al*. (2014), who reported that moderate levels of thymol and carvacrol do not impair carcass yield, even under stress. Notably, the carcass traits of the phytogenic–organic acid group (T3) were comparable to those of the antibiotic group (T2), indicating the potential of these compounds as alternatives to antibiotic growth promoters.

Both antibiotic (T2) and phytogenic (T3) treatments improved foot pad health compared with the control (T1). T2 had the most birds without lesions, significantly exceeding T1 and comparable to T3, while T3 also outperformed T1 (P < 0.05). T2 recorded the fewest mild and severe lesions, with T3 showing a numerical reduction in severe lesions compared with T1. These results indicate that, although antibiotics were most effective, thymol–carvacrol with organic acids is a promising non-antibiotic alternative for reducing lesion prevalence and severity, consistent with reports linking phytogenics to improved integument health via anti-inflammatory, antimicrobial, and gut-modulating effects (Hashemipour *et al*., 2013; Hashemipour *et al*., 2016; Tiyaprasertkul *et al*., 2025).

Meat quality traits, including pH, color, water-holding capacity, sarcomere length, and shear force, were also unaffected by dietary treatments, consistent with previous reports (Guelker *et al*., 2013). This suggests that thymol–carvacrol can be safely used without compromising eating quality.

Lipid oxidation in thigh meat, measured as malondialdehyde (MDA), increased during refrigerated storage (day 1 to day 5, $P < 0.01$), reflecting expected oxidative changes. No significant differences in MDA levels were observed among treatments, indicating that under the current dosage and conditions, thymol–carvacrol did not enhance oxidative stability. Previous studies (Luna *et al*., 2010; Lucera *et al*., 2009) have shown antioxidant benefits at higher dosages or in specific combinations, suggesting that efficacy may depend on dose, formulation, and oxidative challenge level. Serum MDA levels and leukocyte differentials (lymphocytes, monocytes, eosinophils, basophils) were similarly unaffected, in line with earlier findings that systemic antioxidant effects are dose- and formulation-dependent (Hashemipour *et al*., 2013; Li *et al*., 2022).

A key finding of this study was the significant reduction in heterophil-to-lymphocyte (H:L) ratio in both the antibiotic (T2) and phytogenic–organic acid (T3) groups compared with the control (T1), indicating lower physiological and immunological stress. The H:L ratio is a robust biomarker of stress in poultry, with lower values reflecting reduced activation of the hypothalamic–pituitary–adrenal axis and improved immune competence (Gross and Siegel, 1983; Thiam *et al*., 2021). These results mirror those of Banday *et al*. (2025), who found that thyme supplementation reduced serum cortisol and H:L ratio, enhancing stress resilience. The bioactive constituents of thyme—approximately 40% thymol and 15% carvacrol—are likely responsible for these effects through modulation of gut microbiota, enhancement of immune function, and suppression of inflammatory pathways (Azaz *et al*., 2004; Banday *et al*., 2025).

While carcass and meat quality parameters were unaffected, the experimental model—despite the coccidial challenge—may not have been severe enough to elicit differences in performance or yield. Under more demanding commercial conditions with multiple concurrent stressors, the observed reductions in immunological stress could translate into measurable gains in growth, feed efficiency, and carcass quality. The phytogenic–organic acid blend matched the antibiotic in lowering the H:L ratio and improving foot pad health, without compromising carcass or meat quality, highlighting its promise as an antibiotic-free strategy to maintain immune balance, gut integrity, and resilience in broiler production, particularly where stress and lesion risks are elevated.

In summary, dietary supplementation with thymol–carvacrol combined with organic acids did not adversely affect carcass traits or meat quality in broilers under enteric stress. While antibiotics remained the most effective, the phytogenic treatment showed promise as a non-antibiotic alternative for reducing the prevalence and severity of foot pad lesions. Serum malondialdehyde levels and most white blood cell parameters were unchanged; however, the negative control group exhibited higher heterophil percentages and H:L ratios, indicating that both antibiotic and phytogenic treatments mitigated physiological stress. Comparable outcomes between phytogenic- and antibiotic-treated groups further support the potential of thymol–carvacrol with organic acids as a natural replacement for in-feed antibiotics. Additional studies under diverse stress conditions and with optimized inclusion levels are warranted to validate these findings.

Acknowledgements

This work was financially supported by King Mongkut's Institute of Technology Ladkrabang Research Fund (Grant number: KREF016715) and Novus International (Thailand) Co., Ltd. The authors would like to express their sincere gratitude to the livestock farm at the Faculty of Agriculture, Kamphaeng Saen Campus, Kasetsart University, Nakhon Pathom Province, for providing research facilities, and to all staff members for their generous assistance with equipment, technical guidance, and overall support throughout the study.

Conflicts of interest

The authors declare no conflict of interest.

References

Ajin, S. and Thapingkae, W. (2014). Biological properties and function of phytobiotics in animal production. Asian Journal of Animal Sciences, 8:155-167.

Azaz, A. D., Irtem, H. A., Kurkcuoglu, M. and Baser, K. H. C. (2004). Composition and the *in vitro* antimicrobial activities of the essential oils of some Thymus species, Z. Naturforsch, 59:75-80.

Banday, M. T., Wani, M., Alqahtani, F. M., Adriani, L., Alhomrani, M., Adil, S., Alsanie, W. F., Alamri, A. S. and Abdulaziz, O. (2025). Enhancing meat-type chicken performance through Thymus vulgaris leaf powder supplementation by affecting serum lipid profile, stress physiology, immunity, antioxidants, cecal microbiology, and jejunal histomorphology. Archives Animal Breeding, 68:311-323.

Brenes, A. and Roura, E. (2010). Essential oils in poultry nutrition: Main effects and modes of action. Animal Feed Science and Technology, 158:1-14.

Burt, S. (2004). Essential oils: Their antibacterial properties and potential applications in foods: A review. International Journal of Food Microbiology, 94:223-253.

Giannenas, I., Bonos, E., Skoufos, J. and Florou-Paneri, P. (2014). Comparative evaluation of dietary supplementation with oregano, anise and olive oil in growing lambs. Small Ruminant Research, 116:16-22.

Gross, W. B. and Siegel, H. S. (1983). Evaluation of the heterophil/lymphocyte ratio as a measure of stress in chickens. Avian Diseases, 27:972-979.

Grotto, D., Maria, L. S., Valentini, J., Paniz, C., Schmitt, G., Garcia, S. C., Pomblum, V. J. and Rocha, J. B. (2007). Importance of the lipid peroxidation biomarkers and methodological aspects for malondialdehyde quantification. Química Nova, 32:169-174.

Guelker, M. R., Haneklaus, A. N., Brooks, J. C., Carr, C. C., Delmore Jr., R. J., Griffin, D. B., Hale, D. S., Harris, K. B., Mafi, G. G., Johnson, D. D., Lorenzen, C. L., Maddock, R. J., Martin, J. N., Miller, R. K., Raines, C. R., VanOverbeke, D. L., Vedral, L. L., Wasser, B. E. and Savell, J. W. (2013). National beef tenderness survey–2010: Warner-Bratzler shear force values and sensory panel ratings for beef steaks from United States retail and food service establishments. Journal of Animal Science, 91:1005-1014.

Hashemipour, H., Kermanshahi, H., Golian, A. and Veldkamp, T. (2013). Effect of thymol and carvacrol feed supplementation on performance, antioxidant enzyme activities, fatty acid composition, digestive enzyme activities and immune response in broiler chickens. Poultry Science, 92:2059-2069.

Hashemipour, H., Khaksar, V., Rubio, L. A., Veldkamp, T. and Van Krimpen, M. M. (2016). Effect of feed supplementation with a thymol plus carvacrol mixture, in combination or not with an NSP-degrading enzyme, on productive and physiological parameters of broilers fed on wheat-based diets. Animal Feed Science and Technology, 211:117-131.

Kaewhom, P. (2018). Production performance and quality of meat in broilers feed herbal-based additives. Thai Journal of Animal Science, 51:33-44.

Kerekes, E. B., Deák, É., Tako, M., Tserennadmid, R., Petkovits, T., Vágvölgyi, C. and Krisch, J. (2013). Anti-biofilm forming and anti-quorum sensing activity of selected essential oils and their main components on food-related micro-organisms. Journal of Applied Microbiology, 115:933-942.

Li, S. Y., Ru, Y. J., Liu, M., Xu, B., Péron, A. and Shi, X. G. (2012). The effect of essential oils on performance, immunity and gut microbial population in broilers feed diets containing different nutrient density. Journal of Animal and Feed Sciences, 21:244-256.

Lucera, A., Costa, C., Conte, A. and Del Nobile, M. A. (2009). Food applications of natural antimicrobial compounds. Frontiers in Microbiology, 10:1-9.

Luna, A., Lema-Alba, R. C., Dambolena, J. S., Zygadlo, J. A., Labaque, M. C. and Marin, R. H. (2010). Effects of thymol and carvacrol feed supplementation on lipid oxidation in broiler meat. Poultry Science, 89:1016-1022.

Muneer, M., Amin, H., Nadeem, A., Sattar, A., Khan, M. I. and Ali, A. (2021). A comparative study of some hematological parameters of broiler and indigenous breeds of poultry. SVU-International Journal of Agricultural Sciences, 3:189-199.

Nakavisut, S., Phonkerd, K. and Chanchai, W. (2019). Effect of herbal feed additives on performance and blood profiles in broilers. Khon Kaen Agriculture Journal, 47:695-703.

Nwaigwe, C. O., Onyimonyi, A. E., Esonu, B. O. and Okoli, I. C. (2020). Evaluation of the hematological and clinical biochemical markers of stress in broiler chickens. Veterinary World, 13:2294-2300.

Saleh, A. A., Eid, Y. Z., Ebeid, T. A., Ohtsuka, A. and Hayashi, K. (2019). The modification of the muscle fatty acid profile by dietary supplementation with essential oils in broiler chickens. Animal Science Journal, 90:534-543.

Somkuna, S. and Somkuna, T. (2018). Alternatives to antibiotics in poultry feed: Current practices and future perspectives. Journal of Livestock Development, 39:45-52.

Srikijkasemwat, K., Tongda, C., Nukunthon, P., Klompanya, A., Kongrith, C. and Sitthigripong, R. (2025). Oregano (*Origanum vulgare* L.) essential oil as a natural growth promoter in broiler chickens. International Journal of Agricultural Technology, 21:2571-2584.

Thiam, M., Barreto Sanchez, A. L., Zhang, J., Zheng, M., Wen, J., Zhao, G. and Wang, Q. (2021). Association of heterophil/lymphocyte ratio with intestinal barrier function and immune response to Salmonella enteritidis infection in chicken. Animals, 11:3498.

Tiyaprasertkul, P., Phungkeha, P., Srikijkasemwat, K., Philatha, A., Rassmidatta, K., Ruangpanit, Y., Siwapirunthep, P., Yan, F., Romero-Sanchez. and Chaosap, C. (2025). Thymol-carvacrol supplementation in broilers: impact on performance, blood biomarkers, and gut health. International Journal of Agricultural Technology, 21:741-752.

Ultee, A., Bennik, M. H. J. and Moezelaar, R. (2002). The phenolic hydroxyl group of carvacrol is essential for action against the food-borne pathogen Bacillus cereus. Applied and Environmental Microbiology, 68:1561-1568.

Voitsekhivska, O., Polishchuk, S. and Mykhailenko, N. (2021). Broiler meat quality as affected by natural feed additives. Ukrainian Journal of Ecology, 11:398-403.

Windisch, W., Schedle, K., Plitzner, C. and Kroismayr, A. (2008). Use of phytogenic products as feed additives for swine and poultry. Journal of Animal Science, 86:E140-E148.

(Received: 26 August 2025, Revised: 30 December 2025, Accepted: 11 January 2026)