



Dietary supplementation of formic acid and thymol as an antimicrobial alternative improved production, gut health and carcass traits in turkeys

A. CHAMPATI, S.K. BHANJA*, A.S. YADAV, J.J. ROKADE, DIVYA, A. BISWAS, S.K. SAHA, M. SINGH, R.K. AGRAWAL AND N. NAYAK

ICAR- Central Avian Research Institute, Izatnagar, Bareilly, U.P., India

(Received on July 31, 2023; accepted for publication on September 05, 2023)

ABSTRACT

Champati, A., Bhanja, S.K., Yadav, A.S., Rokade, J.J., Divya, Biswas, A., Saha, S.K., Singh, M., Agrawal, R.K. and Nayak, N. 2024. Dietary supplementation of formic acid and thymol as an antimicrobial alternative improved production, gut health and carcass traits in turkeys. *Indian Journal of Poultry Science* 59(1): 15-21.

The consumers are becoming more health conscious and demand for safer food products, alternatives such as organic acid and essential oils are replacing the antibiotic growth promoters (AGP). The study evaluated the efficacy of dietary formic acid (FA) and thymol (Th) on growth, gut morphology, immunity and carcass traits in turkey birds. The CARI VIRAT turkey poults (n=256) were randomly distributed into eight groups having 4 replicates each of 8 birds per replicate: T1(Basal diet: BD), T2 (BD+AGP), T3 (BD+FA 2.5ml/kg), T4 (BD+FA 5 ml/kg), T5 (BD+FA 7.5ml/kg), T6 (BD+Th 120mg/kg), T7 (BD+Th 240mg/kg) and T8 (BD+Th 360mg/kg). The final body weight and cumulative gain and FCR were significantly better for birds under supplementation whereas the mean feed intake across treatments did not show any significant variation. The bursa was significantly heavier in T5 group, whereas T8 group significantly showed best humoral and cell mediated immune responses. The FA and Th supplemented groups had significantly improved villi height, crypt depth and their ratio over the control. The T5 group birds recorded significant reduction in total plate count and *Coliforms* while depicting increment in *Lactobacillus* count. Also, administration of FA and Th improved the cut-up parts, giblet and dressing yield over and above the control. From the study it's concluded that, formic acid 7.5ml/kg and thymol 360 mg/kg supplementations could potentially replace the AGPs in turkey with improved performance, immunity, gut health and carcass yields.

Keywords: Formic acid, Thymol, Growth, Gut health, Turkey

INTRODUCTION

Poultry sector in India is primarily concentrated around chicken, but due to diverse food habit the alternate species like ducks, turkey and quails are now gaining popularity. Turkey (*Meleagris gallopavo*), with the leanest meat among poultry species, can be reared with the sole purpose to increase meat production to meet the demands. In order to improve performance and gut health of poultry, antibiotics have been administered as growth promoters in poultry ration (He *et al.*, 2021). But over the decades, their widespread overuse has brought forth concerns of drug residues in poultry products and also a rising threat of dissemination of microbes resistant to antibiotics, thus rendering them ineffective (Yang *et al.*, 2015). Therefore, to overcome these alarming concerns, the scientific society are now engaged in finding suitable and safe alternatives such like organic acids (OAs) and essential oils (EOs), to replace the AGPs while maintaining the improved production and health in poultry birds (Choi and Kin, 2020). Reports have claimed the positive effect of “Generally recognized as safe” (GRAS) compounds like OAs and EOs on growth, host immunity and gut health (Dhama *et al.*, 2014b). Mechanism via which OAs (formic, butyric, propionic acid) function as antimicrobial in nature and promote growth is by pH modulation in gut of poultry (Paul *et al.*, 2007; Rasschaert *et al.*, 2016).

Similarly, EOs (carvacrol, thymol, eugenol) are volatile bioactive compounds extracted from plants, that encompass beneficial activities such as antioxidant, antimicrobial, immune-modulator and anti-inflammatory (Gopi *et al.*, 2014; Yang *et al.*, 2015). Therefore, the current study was designed with the objective to investigate the effect of dietary supplementation of graded levels of formic acid (FA) and thymol (Th) on growth parameters along with immune response, gut health and carcass traits in turkey birds, as potential and safe alternatives to AGPs.

MATERIALS AND METHODS

Experimental design and germplasm

Total of two hundred fifty-six (256) hatched day-old CARI VIRAT turkey chicks were procured from single hatch, weighed and randomly allotted into 8 treatment groups, with each group having 4 replicates of 8 chicks each. The duration of the study was for 12 weeks from hatch. The chicks were kept in battery brooders up to 6 weeks of age followed by deep litter rearing from 6 to 12 weeks of age. The treatment groups were as follows: T1 (negative control: basal diet: BD only); T2 (positive control: BD+chlortetracycline @ 50mg/kg); T3 (BD+FA 2.5ml/kg); T4 (BD+FA 5 ml/kg); T5 (BD+FA 7.5ml/kg); T6 (BD+Th 120mg/kg); T7 (BD+Th 240mg/kg) and T8 (BD+Th 360mg/kg). Fresh water and mash feed were

*Corresponding author Email: subratcari@gmail.com

administered *ad libitum*. The feed was formulated to meet the requirements as per NRC (1994) as depicted in Table 1. No vaccination was done and no antibiotics (barring T2 AGP) were administered throughout the trial period. Proper light, temperature and general management conditions were kept for birds in all treatment groups.

Table 1: Ingredient and nutrient composition (kg/100kg) of basal diets (NRC, 1994).

Nutrients	Starter phase		Grower phase-1
	0-4 weeks	5-8 weeks	9-12 weeks
ME (Kcal/KG)	2800	2900	3000
Protein %	28.00	26.00	22.00
Lysine %	1.60	1.50	1.30
Methionine %	0.55	0.45	0.40
Calcium %	1.20	1.00	0.85
Phosphorus %	0.60	0.50	0.42
Vitamin A (IU)	5000	5000	5000
Niacin (mg)	60.00	60.00	50.00
Choline (mg)	1600	1400	1100
Feed ingredients (%)			
Maize	49.10	52.45	62.95
Soyabean	40.65	34.05	23.40
Fish meal	8.00	10.00	10.00
Vegetable oil	0.00	1.25	1.35
Di- Calcium phosphate	0.90	0.90	0.90
Lime stone	0.45	0.45	0.45
Trace mineral mixture	0.20	0.20	0.20
A B2 D3	0.10	0.10	0.10
B-complex	0.05	0.05	0.05
Choline Cl	0.05	0.05	0.05
Salt	0.20	0.20	0.20
Lysine	0.20	0.20	0.20
Methionine	0.05	0.05	0.05
Toxin binder	0.05	0.05	0.05

For the zoo-technical parameters, body weight (BW) and mean feed consumption were recorded up to 12 weeks of age and corresponding weight gain (WG) and FCR was calculated. Mortality was recorded daily and at the trial's end, total livability was calculated.

Immunity parameters

The humoral immune response at 6th week of age for 12 turkey birds per treatment were assessed using 1% sheep RBC in standard HA antibody microtitre procedure (Siegel & Gross, 1980). For cell-mediated immune (CMI) response, 12 birds at 6th week of age were subjected to *invivo* cutaneous basophilic hypersensitivity (CBH) test using phytohaemagglutinin-P (Cheng and Lamont, 1988). Relative immune organs (weight index was assessed at 6th and 12th week of age

for 2 birds per replicate per treatment group. The birds were fasted for a period of 12 hours followed by Halal method of slaughtering. The immune organs spleen, bursa of fabricus and thymus were recovered and weighed individually per bird and expressed as relative to live weight [organ weight (g) /live weight (g)].

Gut gross and histomorphological measurements

From the 8 slaughtered birds, each at 6th and 12th week and the intestine was collected in aseptic condition for gross and histomorphometrical measurements. The gross measurements involved the intestinal weight (g) and intestinal length (cm). For histomorphometry, a small portion of jejunal tissue was fixed in 10% neutral-buffered formalin, which was then processed further and embedded in paraffin and cut in fine cross-sectional slides and subjected to Hematoxylin and Eosin (H&E) staining. The stained slides were observed under low power (10x) magnification and parameters including villus height (VH μ m) and width (VW μ m), crypt depth (CD μ m) was measured via Zeiss Primo Star Software and VH: CD was further calculated.

Microbiological analysis of cecal digesta

The caecal digesta samples from 8 slaughtered birds per treatment during 6th and 12th week were collected in sterile tube containing 0.85% NSS, were thoroughly mixed, followed by ten-fold serial dilution, plated on plate count, MacConkey and MRS agar for total plate count (TPC), coliforms and *Lactobacillus spp.* count enumeration as per standard procedure (Miles *et al.*, 1938). The average count per treatment was multiplied with the factor of dilution and was depicted as log₁₀cfu/g of sample.

Carcass traits

At 12th week, eight experimental turkey birds were fasted for 12 h and then weighed, following which they were slaughtered by being exsanguinated underwent post-harvest processing. The cut-up parts in relative to eviscerated weight (g), giblet and dressing yields were calculated in relative to pre-slaughter live weight [organ or part (g) /pre-slaughter/ eviscerated weight (g)].

Statistical analysis

The data recorded was analyzed statistically via one-way ANOVA at 95% confidence interval using suitable statistical packages (SPSS 25.0) as per Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

Body weight and weight gain

Effects of graded doses of FA and Th on BW and weight gain of turkey poults recorded in phases were depicted in Table 2. Until 4th week, the BW of poults across the treatments was comparable. But, the BW at 8th and 12th week, had significant variation with those of treatment groups (T2 to T8) weighed heavier than control (T1). Among the FA and Th groups, the final BW of

Table 2: Effect of formic acid and thymol dietary supplementation on body weight (g) and WG (g) in turkey poult

Group	Body weight (g)				Body weight gain (g)				Livability (%)
	Hatch wt.	4 th week	8 th week	12 th week	0-4 th week	5-8 th week	9-12 th week	0-12 th week	
T1	48±1	313±4	1080 ^b ±61	1697 ^b ±54	264±3	752±55	623 ^b ±25	1639 ^b ±52	95%
T2	48±1	342±8	1312 ^a ±64	2190 ^a ±54	292±6	949±88	878 ^a ±78	2119 ^a ±59	96%
T3	49±1	335±9	1281 ^a ±46	2157 ^a ±58	285±7	929±56	877 ^a ±18	2090 ^a ±61	96%
T4	48±1	322±16	1225 ^a ±104	1991 ^a ±71	274±16	907±87	737 ^{ab} ±41	1917 ^a ±76	95%
T5	49±1	329±15	1255 ^a ±90	2049 ^a ±93	280±15	929±76	794 ^a ±11	2003 ^a ±92	97%
T6	48±1	317±4	1220 ^a ±103	1963 ^a ±72	268±4	897±102	743 ^{ab} ±71	1908 ^a ±73	95%
T7	49±1	322±4	1240 ^a ±67	2002 ^a ±26	274±3	921±68	761 ^{ab} ±42	1956 ^a ±26	97%
T8	49±1	329±23	1267 ^a ±98	2126 ^a ±94	280±23	936±72	860 ^a ±12	2075 ^a ±88	96%
p-value	0.986	0.700	0.038	0.001	0.758	0.702	0.006	0.001	-

Means with different superscripts (a,b) in same column differ significantly ($P \leq 0.05$)

birds was significantly higher for T3 (2157g) and T8 (2126g), respectively which were at par with AGP fed (2190g) group but higher than control (1697g). The cumulative WG was recorded in birds supplemented with FA and Th, portrayed a significantly ($P \leq 0.05$) higher WG compared to T1 control (1639g), with T3 (2090g) and T8 (2075g), respectively, giving the best results and comparable to AGP fed T2 (2119g) group. Our findings corroborated with past authors (Hashemipour *et al.*, 2013; Feye *et al.*, 2021; Li *et al.*, 2022) who reported significant ($P \leq 0.05$) improvement in final BW and WG in birds supplemented with dietary formic acid and thymol in contrast to the birds fed with no additives.

Feed consumption and FCR

The turkey birds across all treatment groups had different feed additives (AGP, FA and Th) except for control (T1). Table 3 represents the phase wise and cumulative feed intake (g) and FCR in turkey birds. The phase wise and the total feed intake throughout the trial period was comparable across the treatments. FCR across the dietary supplemented groups (T2 to T8) was significantly ($P \leq 0.05$) improved over control (T1) during the trial. The cumulative FCR was significantly ($P \leq 0.05$) improved in T8 (2.91) followed by T3 (2.94) in contrast of T1 (3.5). The outcomes of our study concerning feed consumption and FCR were in tandem with reports

(Chowdhury *et al.*, 2018; Nguyen *et al.*, 2018; Yang *et al.*, 2018; Saleem *et al.*, 2020; Rai *et al.*, 2022) stating the improvement in feed intake and FCR with incorporation of feed additives such as formic acid and thymol, possibly via their properties to enhance palatability, antimicrobial effect on pathogens in gut, causing competitive exclusion with increase of beneficial lactobacilli and upregulation of digestive enzymes. An amalgamation of these activities improves nutrient utilization

Immunity parameters

The effect of FA and Th in-feed on CBH (foot web index), antibody titre (humoral) and relative weight of immune organs of turkey poult is presented in Table 4. The foot web index (mm) in T8 was significantly ($p \leq 0.001$) higher (0.87) followed by T3 group (0.66) than other groups while the lowest foot web index was in control T1 group (0.25). The antibody titre in T8 (4.11) was significantly higher, followed by T4 group (3.84), than other groups, while T1 group (3.04) recorded the lowest antibody titre. The relative weight index of bursa in T8 group (0.28) was significantly higher, followed by T5 (0.26) during 6th week, whereas during 12th week, the bursa weight index was significantly heavier for T5 (0.20), followed by T8 (0.17), than other treatment groups. During both 6th and 12th week, T1 group recorded

Table 3: Effect of formic acid and thymol dietary supplementation on feed intake (g) and FCR in turkey poult

Group	Feed intake (g)				FCR			
	0-4 th week	5-8 th week	9-12 th week	0-12 th week	0-4 th week	5-8 th week	9-12 th week	0-12 th week
T1	521±11	2073±150	3167±230	5761±366	1.97 ^a ±0.03	2.75 ^a ±0.04	5.13 ^a ±0.52	3.50 ^a ±0.14
T2	516±9	2068±183	3692±353	6276±264	1.76 ^b ±0.05	2.18 ^b ±0.03	4.19 ^b ±0.06	2.96 ^b ±0.08
T3	527±15	2014±77	3622±134	6162±189	1.85 ^{ab} ±0.01	2.17 ^b ±0.07	4.13 ^b ±0.12	2.94 ^b ±0.04
T4	519±31	1925±144	3271±136	5715±269	1.89 ^{ab} ±0.01	2.14 ^b ±0.11	4.48 ^{ab} ±0.33	2.97 ^b ±0.03
T5	505±33	2060±125	3365±125	5930±279	1.80 ^b ±0.05	2.23 ^b ±0.08	4.23 ^b ±0.14	2.96 ^b ±0.04
T6	509±12	2086±170	3240±273	5835±219	1.90 ^{ab} ±0.05	2.35 ^b ±0.09	4.38 ^{ab} ±0.08	3.06 ^b ±0.09
T7	496±7	1962±79	3444±134	5902±83	1.81 ^b ±0.02	2.15 ^b ±0.09	4.53 ^{ab} ±0.11	3.02 ^b ±0.07
T8	511±52	2094±66	3436±88	6041±187	1.81 ^b ±0.04	2.26 ^b ±0.13	4.00 ^b ±0.12	2.91 ^b ±0.04
P-value	0.993	0.975	0.585	0.723	0.026	0.001	0.050	0.001

Means with different superscripts (a,b,c,d) in same column differ significantly ($P \leq 0.05$)

Table 4: Effect of formic acid and thymol dietary supplementation on immunity profile (cell mediated, relative immune organs weight % and humoral) of turkey poults during 6th and 12th week of age

Group	HI (SRBC)	CMI	6 th week			12 th week		
	log2Ab	response	Spleen (%)	Bursa (%)	Thymus (%)	Spleen (%)	Bursa (%)	Thymus (%)
T1	3.04 ^c ±0.06	0.25 ^c ±0.12	0.11±0.01	0.16 ^c ±0.01	0.11±0.01	0.07±0.01	0.12 ^b ±0.03	0.02±0.02
T2	3.79 ^{bc} ±0.05	0.70 ^{ab} ±0.06	0.13±0.01	0.22 ^{abc} ±0.02	0.16±0.02	0.08±0.01	0.15 ^{ab} ±0.01	0.03±0.01
T3	3.61 ^{cd} ±0.04	0.66 ^{ab} ±0.08	0.13±0.01	0.22 ^{abc} ±0.04	0.15±0.02	0.09±0.02	0.13 ^{ab} ±0.02	0.04±0.01
T4	3.84 ^b ±0.05	0.48 ^b ±0.07	0.12±0.02	0.21 ^{abc} ±0.01	0.14±0.01	0.08±0.01	0.16 ^{ab} ±0.02	0.03±0.01
T5	3.75 ^{bc} ±0.06	0.63 ^{ab} ±0.04	0.13±0.02	0.26 ^{ab} ±0.03	0.14±0.03	0.09±0.01	0.20 ^a ±0.02	0.04±0.01
T6	3.48 ^d ±0.10	0.50 ^b ±0.06	0.12±0.02	0.19 ^{bc} ±0.01	0.12±0.01	0.10±0.01	0.13 ^{ab} ±0.02	0.03±0.02
T7	3.86 ^b ±0.10	0.62 ^b ±0.07	0.16±0.01	0.22 ^{abc} ±0.03	0.14±0.02	0.09±0.01	0.15 ^{ab} ±0.01	0.03±0.01
T8	4.11 ^a ±0.05	0.87 ^a ±0.10	0.15±0.01	0.28 ^a ±0.02	0.17±0.01	0.09±0.02	0.17 ^{ab} ±0.03	0.04±0.02
P- value	0.000	0.000	0.331	0.032	0.500	0.986	0.039	0.975

Means with different superscripts (a,b,c,d,e) in same column differ significantly (P≤0.05)

the lowest relative bursa weight (0.16 and 0.12). Our findings on humoral and cell mediated response coincided with the findings of past authors (Ghasemi *et al.*, 2014; Hashemipour *et al.*, 2013; Emami *et al.*, 2017; Hassan and Awad, 2017) who reported enhanced immune status with spike in antibody IgG and IgM titres against NDV and SRBC and improved CBH response with upregulation of anti-inflammatory cytokines in birds supplemented with dietary formic acid and thymol. In similar fashion, our results on relative immune organ weight index agreed with Alipour *et al.* (2015) and Li *et al.* (2022) who reported improved immunity response was directly correlated to increase in lymphoid organs' weight.

Gut histomorphological and gross measurements

Outcome of the study concerning efficacy of FA and Th on histomorphometry of jejunum and gross intestine measurements of turkey poults during 6th and 12th week are portrayed in Table 5 and 6, respectively. Intestinal weight (% of total BW) was comparable across treatment groups during both periods. The intestinal length (cm) at 6th week was significantly (P<0.05) higher in T8 group (119.25 cm), followed by T5 (116.00 cm) than other FA and Th based treatments, whereas T1 group

recorded the lowest length (101.25 cm). During 6th and 12th week, villi height, crypt depth, VH:CD ratio and villi width were significantly improved in the dietary FA and Th (T3 to T8) based groups over control (T1). The villi height was highest in T5 (1130.47 µm; 1319.07 µm) group during both periods. The crypt depth was highest in T5 (141.78 µm) group during 12th week. The VH:CD ratio was recorded highest for T8 (10.55) and T7 (9.95) during 6th and 12th week, respectively. All the microstructures in T1 group had least values. The gut microstructure, especially in jejunum are vital indicators of nutrient absorption. The reports of Hashemipour *et al.* (2013); Sikandar *et al.* (2017); Ding *et al.* (2022) and NaveenKumar *et al.* (2022), corroborated with our findings indicating increase in villi height, crypt depth and VH:CD ratio in birds supplemented with FA and Th. Possible strong interactions among gut microflora, beneficial traits of formic acid and thymol (muscle thinning, antioxidant, anti-inflammatory) and intestinal microstructure, influence development of the gut villus-crypt units and mucin producing goblet cells enhance nutrient absorption and promote growth in birds (Du *et al.*, 2016; Emami *et al.*, 2017; Liao *et al.*, 2020).

Table 5: Effect of formic acid and thymol dietary supplementation on gross intestinal and jejunal histomorphological measurements in turkey poults during 6th week of age

Group	Intestinal weight (g)	Intestinal weight (%)	Intestinal length (cm)	Villus Height (µm)	Crypt depth (µm)	VH:CD	Villus width (µm)
T1	32.3 ^b ±1.9	4.35±0.3	101.3 ^c ±1.6	913.1 ^{cd} ±12.0	116.5 ^a ±1.6	8.04 ^d ±0.2	125.1 ^c ±3.8
T2	36.1 ^{ab} ±3.4	4.63±0.8	122.0 ^a ±4.0	1011.6 ^b ±14.9	99.9 ^c ±3.7	10.16 ^{ab} ±0.3	157.4 ^a ±8.0
T3	37.2 ^{ab} ±1.4	4.69±0.3	111.0 ^{bc} ±4.1	957.3 ^{bc} ±26.1	99.8 ^c ±2.3	9.60 ^{ab} ±0.3	145.2 ^{abc} ±4.2
T4	34.7 ^{ab} ±0.4	4.22±0.2	111.0 ^{bc} ±2.5	887.5 ^d ±32.4	104.5 ^{bc} ±2.3	8.51 ^{cd} ±0.4	131.4 ^{bc} ±5.9
T5	40.0 ^a ±1.2	4.80±0.4	116.0 ^{ab} ±2.7	1130.5 ^a ±18.0	113.8 ^{ab} ±1.7	9.94 ^{ab} ±0.2	152.6 ^{ab} ±8.0
T6	37.0 ^{ab} ±2.6	4.70±0.3	114.0 ^{ab} ±3.8	936.7 ^{cd} ±11.2	112.4 ^{ab} ±4.5	8.39 ^{cd} ±0.5	135.6 ^{abc} ±5.8
T7	37.3 ^{ab} ±1.1	4.67±0.3	118.0 ^{ab} ±3.9	1080.6 ^a ±16.7	117.4 ^a ±3.6	9.24 ^{bc} ±0.4	146.1 ^{abc} ±9.1
T8	38.9 ^a ±0.3	4.77±0.2	119.6 ^{ab} ±2.8	1076.2 ^a ±10.9	102.2 ^c ±3.1	10.55 ^a ±0.3	150.9 ^{ab} ±9.6
P- value	0.019	0.938	0.050	0.001	0.001	0.001	0.042

Means with different superscripts (a,b,c,d) in same column differ significantly (P≤0.05)

Table 6: Effect of formic acid and thymol dietary supplementation on gross intestinal and jejunal histomorphological measurements in turkey poult during 12th week of age

Group	Intestinal weight (g)	Intestinal weight (%)	Intestinal length (cm)	Villus Height (µm)	Crypt depth (µm)	VH:CD	Villus width (µm)
T1	68.6 ^b ±3.7	3.99±0.1	146.5±5.5	1031 ^b ±8.8	125.1 ^{ab} ±6.6	8.32 ^b ±0.5	132.8 ^a ±9.1
T2	934.0 ^a ±8.5	4.55±0.1	157.3±2.8	1308 ^a ±64.2	132.5 ^{ab} ±5.4	9.92 ^a ±0.6	150.9 ^{ab} ±10.1
T3	84.1 ^{ab} ±3.4	4.09±0.5	147.3±3.0	1243 ^a ±22.1	130.7 ^{ab} ±1.9	9.51 ^{ab} ±0.1	163.8 ^a ±5.0
T4	78.3 ^{ab} ±7.1	3.95±0.4	147.3±2.2	1107 ^b ±37.3	119.5 ^b ±2.7	9.29 ^{ab} ±0.5	146.3 ^{ab} ±11.7
T5	88.4 ^a ±3.3	4.33±0.4	150.5±6.0	1319 ^a ±25.5	141.8 ^a ±10.9	9.45 ^{ab} ±0.7	160.1 ^a ±6.5
T6	76.6 ^{ab} ±1.5	4.01±0.2	147.8±6.5	1127 ^b ±48.8	127.5 ^{ab} ±6.4	8.86 ^{ab} ±0.3	144.9 ^{ab} ±3.0
T7	89.8 ^a ±6.3	4.32±0.7	152.5±4.7	1294 ^a ±22.4	130.2 ^{ab} ±0.9	9.95 ^a ±0.2	134.9 ^{bc} ±4.9
T8	87.2 ^a ±5.8	4.08±0.3	158.5±1.9	1276 ^a ±39.6	132.5 ^{ab} ±3.2	9.63 ^{ab} ±0.2	158.1 ^{ab} ±3.7
P- value	0.017	0.955	0.363	0.000	0.041	0.031	0.009

Means with different superscripts (a,b,c) in same column differ significantly (p≤0.05)

Microbiological analysis of cecal digesta

Enumeration of the microbes from cecal digesta (log 10 cfu/g) during 6th and 12th week, affected by in-feed formic acid and thymol administered to turkey is depicted in Table 7. For both periods, a very significant (p≤0.001) reduction in TPC and coliforms seen in T5 and T8 groups in contrast to T1. Also, *Lactobacillus* count significantly (p≤0.05) improved in T5 and T8. During both the periods, T1 group recorded the poorest results as compared to other groups. Results of our study were in agreement with authors (Du et al., 2016, Emami

et al., 2017; Nguyen et al., 2018; Pourreza et al., 2023) suggesting significant spike of the *Lactobacilli*: *E.coli* ratio, reduction of coliform and TPC in gut which might be the cause for the better performance via competitive exclusion, affected due to strong antimicrobial action of formic acid and thymol.

Cut-up parts, giblet and dressing yield

The yields of the carcass cut-up parts, giblet and dressing % as affected by dietary formic acid and thymol in turkey are portrayed in Table 8. The carcass traits were positively influenced by dietary feed additives (FA

Table 7: Effect of formic acid and thymol dietary supplementation on caecal microflora (log10 cfu/g) of turkey poult during 6th and 12th week of age

Group	Total plate count		Coliform		Lactobacillus	
	6 th week	12 th week	6 th week	12 th week	6 th week	12 th week
T1	8.10 ^a ±0.01	8.06 ^a ±0.01	8.06 ^a ±0.01	8.03 ^a ±0.01	5.89 ^a ±0.00	5.92 ^b ±0.01
T2	7.93 ^{cde} ±0.03	8.01 ^b ±0.01	7.87 ^d ±0.03	7.97 ^b ±0.02	5.92 ^{bc} ±0.01	5.93 ^{ab} ±0.02
T3	8.02 ^b ±0.01	7.99 ^{bc} ±0.01	7.98 ^b ±0.02	7.97 ^{bc} ±0.01	5.92 ^{bc} ±0.01	5.94 ^{ab} ±0.02
T4	7.96 ^{bcd} ±0.02	7.96 ^{cde} ±0.02	7.94 ^{bcd} ±0.02	7.93 ^{bcd} ±0.01	5.93 ^{ab} ±0.01	5.96 ^{ab} ±0.01
T5	7.89 ^e ±0.03	7.92 ^{ef} ±0.01	7.79 ^e ±0.03	7.89 ^d ±0.01	5.96 ^a ±0.01	5.99 ^a ±0.03
T6	7.97 ^{bc} ±0.01	7.99 ^{bcd} ±0.02	7.96 ^{bc} ±0.02	7.97 ^{bc} ±0.01	5.91 ^{bc} ±0.01	5.92 ^b ±0.03
T7	7.93 ^{cde} ±0.01	7.95 ^{def} ±0.01	7.90 ^{cd} ±0.04	7.93 ^{cd} ±0.01	5.92 ^{bc} ±0.02	5.93 ^{ab} ±0.02
T8	7.90 ^{de} ±0.04	7.91 ^f ±0.01	7.88 ^{cd} ±0.02	7.89 ^d ±0.03	5.93 ^{bc} ±0.01	5.94 ^{ab} ±0.02
P- value	0.001	0.001	0.001	0.001	0.017	0.048

Means with different superscripts (a,b,c,d,e,f) in same column differ significantly (P≤0.05)

Table 8: Effect of formic acid and thymol dietary supplementation on carcass traits of turkey poult

Group	Thigh %	Drumstick %	Breast %	Back %	Neck %	Wings %	Giblet %	Dressing %
T1	13.2 ^b ±0.4	13.0 ^{ab} ±0.6	25.9 ^{bc} ±0.3	20.7±0.4	7.1±0.3	13.2±0.2	5.5 ^b ±0.1	82.9 ^d ±0.2
T2	14.3 ^a ±0.37	13.2 ^{ab} ±0.4	28.1 ^a ±0.9	21.6±0.3	7.6±0.4	13.8±0.3	6.5 ^a ±0.2	84.5 ^{cd} ±0.2
T3	14.2 ^a ±0.3	13.2 ^{ab} ±0.2	28.0 ^a ±0.3	22.4±0.6	7.3±0.5	13.7±0.4	6.2 ^a ±0.3	85.6 ^{bc} ±0.6
T4	13.7 ^{ab} ±0.3	12.8 ^b ±0.1	26.2 ^{bc} ±1.0	21.2±0.2	7.4±0.3	13.7±0.1	5.8 ^{ab} ±0.2	83.3 ^d ±0.4
T5	14.7 ^a ±0.2	13.2 ^{ab} ±0.4	26.6 ^{a-c} ±0.4	23.1±0.5	7.4±0.1	13.9±0.3	6.2 ^a ±0.3	87.8 ^a ±0.6
T6	14.7 ^{ab} ±0.3	12.25 ^b ±0.27	25.0 ^c ±0.4	21.2±0.5	7.4±0.1	13.8±0.4	5.8 ^{ab} ±0.1	85.5 ^{bc} ±0.9
T7	14.2 ^a ±0.3	13.37 ^{ab} ±0.45	27.3 ^{ab} ±0.4	23.0±0.7	7.6±0.2	14.0±0.6	6.0 ^{ab} ±0.2	86.4 ^{a-c} ±1.1
T8	14.5 ^a ±0.2	14.20 ^a ±0.53	26.7 ^{ab} ±0.6	23.3±2.0	7.5±0.18	13.4±0.3	6.1 ^{ab} ±0.3	86.5 ^{ab} ±0.3
P-value	0.050	0.026	0.002	0.248	0.954	0.780	0.011	0.000

Means with different superscripts (a,b,c,d) in same column differ significantly (P≤0.05)

and Thymol). The thigh, drumstick and breast yields were significantly ($P \leq 0.05$) heavier for T5 and T8 group in contrast to T1, whereas back, neck and wings yield were comparable among treatment groups. The T5 and T8 also yielded significantly ($P \leq 0.05$) better giblet and dressing %. These results were coinciding with past carcass studies (El-Ghousein and Al-Beitawi, 2009; Ragaa et al., 2016; Cázares-Gallegos et al., 2019; Al-Ghamdi, 2022; Rai et al., 2022) that reported positive and notable improvements in carcass cut up parts, giblet and dressing yield in birds supplemented with formic acid and thymol which was correlated with improved growth and muscle mass in the treatments, as compared to control group.

CONCLUSIONS

From this study, it is inferred that varied levels of in-feed supplementation of formic acid 7.5 ml/kg and thymol 360 mg/kg in turkey birds can improve their growth, boost their immune responses, promote a healthy gut resulting better utilization and absorption of nutrients and improved carcass and dressing yields. Further, formic acid and thymol can effectively be used to replace AGPs in turkey birds.

CONFLICT OF INTEREST

The authors declare no conflict of interest

REFERENCES

- Al-Ghamdi, E.S. 2022. Growth performance, carcass characteristics, and blood biochemical indices of broilers affected by dietary organic acids blend's supplementation. *Animal Biotechnology*, 1-6.
- Alipour, F., Hassanabadi, A., Golian, A. and Nassiri-Moghaddam, H. 2015. Effect of plant extracts derived from thyme on male broiler performance. *Poultry Science*, **94**(11): 2630–2634.
- Cázares-Gallegos, R., Silva-Vázquez, R., Hernández-Martínez, C.A., Gutiérrez-Soto, J.G., Kawas-Garza, J. R., Hume, M.E., and Méndez-Zamora, G.M. 2019. Performance, carcass variables, and meat quality of broilers supplemented with dietary Mexican oregano oil. *Brazilian Journal of Poultry Science*, **21**(1): 1-10.
- Cheng, S. and Lamont S.J. 1988. Genetic analysis of immunocompetence measures in a white leghorn chicken line. *Poultry Science*, **67**(7): 989-995.
- Choi, J. and Kim, W.K. 2020. Dietary application of tannins as a potential mitigation strategy for current challenges in poultry production: A review. *Animals*, **10**(12): 2389
- Chowdhury, S., Mandal, G.P. and Patra, A.K. 2018. Different essential oils in diets of chickens: 1. Growth performance, nutrient utilisation, nitrogen excretion, carcass traits and chemical composition of meat. *Animal Feed Science and Technology*, **236**: 86-97.
- Dhama, K., Tiwari, R., Chakraborty, S., Saminathan, M., Kumar, A., Karthik, K., Wani, M.Y., Amarpal, Singh, S.V. and Rahal, A. 2014. Evidence based antibacterial potentials of medicinal plants and herbs countering bacterial pathogens especially in the era of emerging drug resistance: An integrated update. *International Journal of Pharmacology*, **10**(1): 1-43.
- Ding, Y., Hu, Y., Yao, X., He, Y., Chen, J., Wu, J., Wu, S., Zhang, H., He, X. and Song, Z. 2022. Dietary essential oils improves the growth performance, antioxidant properties and intestinal permeability by inhibiting bacterial proliferation, and altering the gut microbiota of yellow-feather broilers. *Poultry Science*, **101**(11): 102087.
- Du, E., Wang, W., Gan, L., Li, Z., Guo, S. and Guo, Y. 2016. Effects of thymol and carvacrol supplementation on intestinal integrity and immune responses of broiler chickens challenged with *Clostridium perfringens*. *Journal of Animal Science and Biotechnology*, **7**(1): 1-10.
- El-Ghousein, S.S. and Al-Beitawi, N.A. 2009. The effect of feeding of crushed thyme (*Thymus vulgaris* L) on growth, blood constituents, gastrointestinal tract and carcass characteristics of broiler chickens. *The Journal of Poultry Science*, **46**(2): 100-104.
- Emami, N.K., Daneshmand, A., Naeini, S.Z., Graystone, E.N. and Broom, L.J. 2017. Effects of commercial organic acid blends on male broilers challenged with *E. coli* K88: Performance, microbiology, intestinal morphology, and immune response. *Poultry Science*, **96**(9): 3254-3263
- Feye, K.M., Dittoe, D.K., Jendza, J.A., Caldas-Cueva, J.P., Mallmann, B.A., Booher, B., Tellez-Isaias, G., Owens, C.M., Kidd, M.T. and Ricke, S.C. 2021. A comparison of formic acid or monoglycerides to formaldehyde on production efficiency, nutrient absorption, and meat yield and quality of Cobb 700 broilers. *Poultry Science*, **100**(12):101476.
- Ghasemi, H.A., Akhavan-Salamat, H., Hajkhodadadi, I. and Khaltabadi-Farahani, A.H. 2014. Effects of dietary organic acid blend supplementation on performance, intestinal morphology and antibody-mediated immunity in broiler chickens. *Acta Advances in Agricultural Sciences*, **2**(10): 64-74.
- Gopi, M., Karthik, K., Manjunathachar, H. V., Tamilmahan, P., Kesavan, M., Dashprakash, M., Balaraju, B.L. and Purushothaman, M.R. 2014. Essential oils as a feed additive in poultry nutrition. *Advances in Animal and Veterinary Sciences*, **2**(1): 1-7.
- 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**(8): 2059-2069.
- Hassan, F.A. and Awad, A. 2017. Impact of thyme powder (*Thymus vulgaris* L.) supplementation on gene expression profiles of cytokines and economic efficiency of broiler diets. *Environmental Science and Pollution Research*, **24**: 15816-15826.
- He, Y., Liu, X., Dong, Y., Lei, J., Ito, K. and Zhang, B. 2021. *Enterococcus faecium* PNC01 isolated from the intestinal mucosa of chicken as an alternative for antibiotics to reduce feed conversion rate in broiler chickens. *Microbial Cell Factories*, **20**(1):1-14.
- Li, Z., Jin, X., Wu, Q., Long, L., Li, Y., Zhang, Q., Liu, A., Chen, X., Geng, Z. and Zhang, C. 2022. Effects of encapsulated thymol and carvacrol mixture on growth performance, antioxidant capacity, immune function and intestinal health of broilers. *Italian Journal of Animal Science*, **21**(1): 1651-1659.
- Liao, X., Shao, Y., Sun, G., Yang, Y., Zhang, L., Guo, Y., Luo, X. and Lu, L. 2020. The relationship among gut microbiota, short-chain fatty acids, and intestinal morphology of growing and healthy broilers. *Poultry Science*, **99**(11): 5883-5895.

- Miles, A.A., Misra, S.S. and Irwin, J.O. 1938. The estimation of the bactericidal power of the blood. *Epidemiology and Infection*, **38**(6): 732-749.
- National Research Council (US). National Committee on Science Education Standards, 1994. *National Science Education Standards: Draft for Review and Comment Only*. National Research Council.
- Naveenkumar, S., Karthikeyan, N., Veeramani, P. and Thangapandian, M. 2022. Effect of organic acid salts as an alternative to antibiotic growth promoters on growth performance and intestinal morphometry of commercial broiler chicken. *Indian Journal of Poultry Science*, **57**(1): 75-82.
- Nguyen, D. H., Lee, K.Y., Mohammadigheisar, M. and Kim, I.H. 2018. Evaluation of the blend of organic acids and medium-chain fatty acids in matrix coating as antibiotic growth promoter alternative on growth performance, nutrient digestibility, blood profiles, excreta microflora, and carcass quality in broilers. *Poultry Science*, **97**(12): 4351-4358.
- Pourreza, A., Javandel, F., Seidavi, A. and Franco-Robles, E. 2023. The effect of dietary organic acids on performance, carcass characteristics, immunity, blood constituents and ileal microflora of broiler chickens. *Animal Science Papers and Reports*, **41**(1).
- Ragaa, N.M., Korany, R.M. and Mohamed, F.F. 2016. Effect of thyme and/or formic acid dietary supplementation on broiler performance and immunity. *Agriculture and Agricultural Science Procedia*, **10**: 270-279.
- Rai, A., Bhattacharyya, A., Shukla, P.K., Kumar, M., Jadhav, P.R. and Raghav, H. 2022. Effect of dietary supplementation of oregano and thyme oil on the growth performance, development of lymphoid and digestive organs and carcass quality traits of Turkey poults. *Indian Journal of Poultry Science*, **57**(1): 35-42.
- Saleem, K., Saima, R.A., Pasha, T.N., Hayat, Z. and Hayat, Z. 2020. Effects of dietary organic acids on performance, cecal microbiota, and gut morphology in broilers. *Tropical Animal Health and Production*, **52**(6): 3589-3596.
- Siegel, P.B. and Gross W.B. 1980. Production and persistence of antibodies in chickens to sheep erythrocytes. Directional selection. *Poultry Science*, **59**(2): 1-5.
- Sikandar, A., Zaneb, H., Younus, M., Masood, S., Aslam, A., Khattak, F., Ashraf, S., Yousaf, M. S. and Rehman, H. 2017. Effect of sodium butyrate on performance, immune status, microarchitecture of small intestinal mucosa and lymphoid organs in broiler Chickens. *Asian-Australasian Journal of Animal Sciences*, **30**(5): 690-699.
- Snedecor, G.W. and Cochran, W.G. 1994. Statistical methods. 8th Edn. Oxford and IBH Publishing House, Calcutta.
- Yang, C., Chowdhury, M., Huo, Y. and Gong, J. 2015. Phytogetic compounds as alternatives to in-feed antibiotics: Potentials and challenges in application. *Pathogens*, **4**: 137–156
- Yang, X., Xin, H., Yang, C. and Yang, X. 2018. Impact of essential oils and organic acids on the growth performance, digestive functions and immunity of broiler chickens. *Animal Nutrition*, **4**(4): 388-393.