Original Research

Journal of Advanced Veterinary Research
(2026) Volume 16, Issue 4, 525-530

Role of phytogenic feed additives in modulating *NRF-2/IL-1β* signaling, immune response, and growth performance in cold-stressed broiler chickens

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ARTICLE INFO

Received: 08 April 2026

Accepted: 15 June 2026

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Keywords:

Antibody titer, Antioxidant, *Cytokines*, *Herb-ALL™ RESP*, inflammation, Production.

ABSTRACT

Cold stress is a significant environmental factor negatively affecting broiler performance, immune competence and respiratory health. The aim of this study was to investigate growth promoting, immunomodulatory, antioxidant and anti-inflammatory effects of phytogenic feed additive (PFA) (Herb-ALL™ RESP) on broiler chickens subjected to cold stress in winter season. A total of 480 one-day-old, Ross 308 broiler chicks were assigned to four groups (120 birds each; four replicates of 30 birds): NT group (control): Normal temperature + basal diet without dietary PFA; NT+PFA group: Normal temperature with 1 kg/ton feed dietary PFA (Herb-ALL™ Resp); LT group: Low temperature without dietary PFA; LT+PFA group: Low temperature with 1 kg/ton feed dietary PFA. Cold stress showed compromised broiler growth performance, decrease antibody titer against Newcastle disease virus (NDV), with down regulation of antioxidant *NRF-2* gene and upregulation of pro inflammatory cytokines *IL-1β* gene in tracheal and lung tissue. The PFA supplementations markedly improved broiler body weight, feed conversion ratio, European production efficiency factor, immune response against NDV and antioxidant status (↑*NRF-2* gene expression), and decrease in inflammatory conditions (↓ *IL-1β* gene expression in both NT and LT conditions, with a decrease in weekly feed intake in LT environment only. These findings highlight the potential of PFA as a natural strategy to improve the broiler productivity and health under cold stress conditions.

Introduction

Cold stress is a physical environmental stressor affecting broiler health and survival (Hu *et al*., 2021). It also affects the growth performance and welfare of broiler chickens (Yang *et al*., 2014). Cold stress-induced homeostatic dysfunctions continue to pose a hazard, especially for young broiler chickens (Zhou *et al*., 2021). Broiler chickens gradually develop temperature regulation after hatching, making newly hatched chicks highly vulnerable to cold due to their heat-producing organs are still immature (Mujahid *et al*., 2009). It has been reported that cold stress lowers weight gain, feed efficiency, and causes early mortality in broilers (Moreira *et al*., 2024). Additionally, Cold stress results in immunosuppression and reduces the lymphocytes in broilers (Yang *et al*., 2014). Slota *et al*., (2015) reported that cold stress activates the hypothalamic-pituitary-thyroid axis, increasing corticosterone levels, which are linked to stress and immunosuppression. Additionally, it causes oxidative stress, inflammation, and immune dysfunction in broilers (Su *et al*., 2018; Xue *et al*., 2021).

The cost of heating fuel significantly affects the poultry industry's profit margins, posing challenges to broiler production during winter (Tsiouris *et al*., 2015). Traditional methods to address low-temperature stress are costly, energy-intensive, and detrimental to the environment (Damaziak *et al*., 2021; Suarez *et al*., 2021). Using phytogenic feed additives (PFA) has been one of the common trends to enhance the low-temperature stress on poultry in recent years (Da Rosa *et al*., 2020; Hu *et al*., 2021). Dietary supplementation with PFAs enhances broiler growth, health, and immunity, helping to reduce the negative effects of environmental stressors (Saleh *et al*., 2018; Abd El-Ghany, 2025)

Emblica officinalis, known as *Amla* or Indian gooseberry, is a monoecious deciduous tree from the Euphorbiaceae family, found across India, Pakistan, Nepal, Sri Lanka, Southeast Asia, China, and Malaysia at elevations of 610-1390 meters (Sahni, 1998; Bhattacharya *et al*., 2007). *Amla* is explored as PFA in livestock and poultry because its medicinal properties improve health and prevent respiratory, digestive, inflammatory, and blood disorder (Kumar *et al*., 2018). *Emblica officinalis* contains abundant minerals, amino acids, ascorbic acid, tannins, flavonoids, alkaloids, and polyphenolic compounds (Yokozawa *et al*., 2007; Zhang *et al*., 2003). These bioactive constituents can be effectively utilized to enhance broiler productivity (Patel *et al*., 2016). Also is associated with enhanced immune responses in broilers, including increased lymphocyte counts and antibody production (Mandal *et al*., 2017). Furthermore, it activates antioxidant enzyme systems, which protect tissues from free radicals and improve cell survival, thereby reducing inflammation-related stress in poultry (Kazal *et al*., 2023; Lee *et al*., 2023)

Cinnamon, belonging to the Cinnamomum genus of the Lauraceae family, includes 250–300 aromatic evergreen species, though only a few, like *Cinnamomum cassia* (Chinese cinnamon), are globally significant (Paranagama *et al*., 2020) *Cinnamomum cassia* contains significant amounts of natural antioxidants, antimicrobials, and anti-inflammatory components such as cinnamaldehyde, flavonoids, curcuminoids, coumarins, tannins, alkaloids, xanthones, terpenoids, phenolics, and other compounds (Ribeiro-Santos *et al*., 2017; Liyanage *et al*., 2021). *Cinnamomum cassia* serves as a natural alternative to antibiotic growth promoters in broilers, improving growth performance, strengthening immune function, and promoting gut health (Singh *et al*., 2014). *Cinnamomum cassia* compounds, including cinnamaldehyde, cinnamic acid, and eugenol, promote *NRF-2* activation, suppress NF-κB, and strengthen antioxidant and anti-inflammatory responses (Almoiliqy *et al*., 2020; Choi *et al*., 2020). The systemic immunostimulants, antioxidants, and anti-inflammatory properties of *Cinnamomum cassia* suggest their potential benefits in broiler chickens against environmental stressors. Therefore, our study aimed to investigate how dietary supplementation of PFA (Herb-ALL™ RESP) containing *Cinnamomum cassia* and *Emblica officinalis* affects growth performance, immune response, and *NRF-2/IL-1β* gene expression in the tracheal and lung tissue of broiler chickens under cold stress.

ISSN: 2090-6277/2090-6269/

Materials and methods

The experiment was carried out in the Poultry Research Unit, Department of Veterinary Hygiene and Management, Faculty of Veterinary Medicine, Cairo University, Egypt. The study was approved by the Institutional Animal Care and Use Committee (IACUC), Faculty of Veterinary Medicine, Cairo University, Egypt (Ethical reference No: Vet CU081020251239), and in accordance to ARRIVE guidelines.

Husbandry, experimental design, and diets

The experiment involved 480 one-day-old, Ross 308 broiler chicks obtained from a commercial hatchery. The chicks were weighed at the experiment's onset. The design was completely randomized, with two rearing temperatures from the first week till 35 day (end of experiment), (normal or low temperature) and two dietary treatments (basal diet or diet fortified with 1kg/ton feed Phytogenic feed additive (PFA) "Herb-ALL™ RESP" preparation), which creates four groups [120 birds/ group with four replicates of 30 chicks each (stocking density: 10 birds/ m2)]: NT group (control): Normal temperature + basal diet without dietary PFA; NT+PFA group: Normal temperature + 1 kg/ton feed PFA (Herb-ALL™ Resp); LT group: Low temperature without PFA; LT+PFA group: Low temperature + 1 kg/ton feed PFA. In the normal temperature groups, chicks were raised at 33°C at the 1st d of age which decreased gradually till reach 24°C at 21 d (0.5°C/d) that maintained for the rest of the experiment with 55-60% relative humidity (RH), while in the low temperature groups the chicks was kept in ambient winter temperature ranged from 18 to 22°C during the experimental period, with 55- 60% relative humidity (RH). The lighting program was 24 h. light for the first three days, then 23L: 1D for the rest of the trial (Lan *et al*., 2020).

The basal diet (A three-phase corn-soybean-based diet) was formulated (Table 1) to fulfill Ross 308 broilers' nutritional requirements (Aviagen, 2014) , while the other diet was fortified with the (1kg/ton feed) PFA "Herb-ALL™ RESP" preparation (Life Circle Nutrition AG, Hämmerli 2d, 8855, Wangen SZ, Switzerland), according to the manufacturer's recommendation. *Cinnamomum cassia* and *Emblica officinalis* are two of the pre-standardized, tested herbs in the polyherbal preparation "Herb-ALL™ RESP". The herbal composition (as provided by the manufacturer) was as follows: polyphenols: 7.50 g (g GAE (gallic acid equivalents) /100g), 18.5% crude fiber, 5.2% crude protein, 1.8% crude fat, 7.6% crude ash, 0.02% sodium, 8.5% moisture. Herb-ALL™ RESP was added to the diet across all growth phases (days 1–35), with birds fed a mash diet to ensure proper mixing. Feed and water were available ad libitum.

Growth performance

Weighing the birds and feeds at the start and weekly interval were carried out to determine the average live body weight (BW), body weight growth (BWG), feed intake (FI), and feed conversion ratio (FCR) (Bawish,*et al*., 2024). The number of dead birds daily was estimated. The FI and subsequently FCR were adjusted according to the mortality rate. The European Production Efficiency Factor (EPEF) was calculated (Huff *et al*., 2013) as follows:

EPEF = (livability × live weight (kg) / (age in days × FCR) × 100

Assessment of the ND vaccine's immune status

To assess immunity to Newcastle Disease Virus (NDV), the haemagglutination inhibition (HI) assay was carried out as recommended by WOAH (2023). In brief, 25 µl of each serum sample underwent two-fold serial dilution in 96-well microplates. This was then combined with 25 µl of NDV-LaSota antigen, which contained four haemagglutination units. The solutions were gently mixed and incubated at room temperature for 20 minutes to facilitate antigen-antibody binding. Subsequently, 25 µl of a 1% suspension of chicken red blood cells was added to each well. The plates were then incubated for 30 to 45 minutes, and the HI titers were determined and reported as log2 values (Alaa *et al*., 2024).

Table 1. Ingredients and nutrient composition of basal diets for each growing period.

Ingredients%	Starter (0 to 14 days)	Grower (15 to 28 days)	Finisher (29 to 35 days)
Yellow corn	55	59.2	65.04
Soybean meal 46%	39.72	36	30.7
Soy oil	1	1.35	1.15
Limestone	1.25	1	0.95
L. Lysine HCl 78.5%	0.24	0.17	0.2
Monocalcium phosphate	1.42	1	0.69
Salt	0.2	0.2	0.2
Sodium bicarbonate	0.25	0.25	0.25
L. Therionine 98.5%	0.15	0.1	0.1
DL. Methionine 99%	0.23	0.21	0.2
Vitamin mineral premix[1]	0.3	0.3	0.3
Anticoccidial	0.05	0.05	0.05
Choline chloride 60%	0.05	0.05	0.05
Mycotoxin binder	0.1	0.1	0.1
Total	100	100	100
Chemical analysis			
ME (Kcal/kg)	2920	2995	3240
Crude Protein (%)	23	21.5	19.5
Crude Fiber (%)	2.76	2.71	2.63
Calcium (%)	0.95	0.75	0.65
P. Available (%)	0.4	0.32	0.26

[1]Vitamin and mineral mixture contained: 13000000 IU vitamin A; 80000 mg vitamin E; 6000000 IU vitamin D3; 4000 mg vitamin K; 5000 mg vitamin B1; 9000 mg vitamin B2; 5000 mg vitamin B6; 35 mg vitamin B12; 20000 mg pantothenic acid; 2000 mg Folic acid; 70000 mg Nicotinic acid; 250 mg Biotin; 100000 mg Zinc oxide; 400000 mg Manganese oxide; 1000 calcium Iodide; 15000 mg Copper sulphate; 350 mg Selenium selenite; 50000 mg ferrous sulphate.

RNA extraction and qRT-PCR for NRF-2 and IL-1β

Samples were subjected to RNA isolation using the Qiagen mini RNeasy extraction kit following the manufacturer's instructions (Mesalam *et al*., 2021). Purity and concentration of the extracted RNA were determined using NanoDrop at 260 and 280 nm. The complementary DNA (cDNA) was synthesized using the Revert Aid First Strand cDNA Synthesis Kit from Thermo Scientific, following the manufacturer's instructions (Fermentas, Lithuania). Primers for target gene expression analysis were designed using Gallus gallus sequences from GenBank with the Primer3 tool. Real-time PCR analysis utilized SYBR Green PCR Master Mix and the ABI Prism StepOnePlus Real-Time PCR System from Applied Biosystems, following the manufacturer's instructions (Thermoscientific Cat number: 4309155). Each PCR reaction was performed twice on each sample (Ibrahem and Ibrahim, 2014). The expression levels of *NRF-2* and *IL-1β* were normalized to that of the housekeeping gene beta-actin. The modified fold change method was used to analyze the gene expression data, as described by Gamal and Ibrahim method (2024).

Statistical analysis

Data were analyzed using 2-way ANOVA as a completely randomized design using PASW Statistics, Version 24.0. software (SPSS Inc., Armonk, NY, USA). The statistical model included the main effects of Temperature, PFA level, and their interaction. The results were presented as Mean±SE. Tukey's post hoc test was employed to make multiple comparisons. The graphs comparing Mean±SE of different groups were created using

GraphPad Prism version 6.00 (GraphPad Software, San Diego, CA, USA). Boxplot was created with the ggplot2 and ggpubr packages of R version 4.4.3 (Kassambara *et al*., 2023; Wickham *et al*., 2025). The significance level was set at $p < 0.05$.

Results

Productive performance

Tables 2 and 3 present the effects of cold stress, PFA supplementation, and their interaction on broiler growth performance parameters. Exposure of birds to cold stress negatively affects their growth performance, which observed by a significant reduction ($p<0.05$) in body weight (BW) from day 7 till 35 of age, in body weight gain (BWG) at days 14, 21, 35, and the overall period, and the EPEF in the LT group compared to the NT group. On the other hand, there was a significant ($p<0.05$) increment in the weekly feed intake (WFI) in the LT group at days 7, 14, 35 and all over the trial period, in the FCR at days 7, 14, 21, 25 and the overall period, and in mortality compared to the NT group.

The dietary PFA (1 kg/ton feed) markedly improved the growth per-

Table 2. Body weight and body weight gain of broiler chickens supplemented with PFA under low temperature conditions.

Parameters	Treatments						
	NT	NT+PFA	LT	LT+PFA	P-value Temperature (T)	P-value Additive (A)	P-value Interaction (T x A)
BW (g)							
7 days	139.33±1.45^{a}	140.63±2.24^{a}	134.00±1.58^{b}	140.50±1.17^{a}	0.13	0.037*	0.14
14 days	386.25±1.05^{c}	427.25±1.79^{a}	371.63±1.68^{d}	401.50±3.75^{b}	0.000*	0.000*	0.003*
21 days	765.88±1.64^{c}	823.75±1.25^{a}	711.50±1.21^{d}	745.63±1.70^{b}	0.000*	0.000*	0.000*
28 days	1368.38±3.01^{b}	1424.75±2.24^{a}	1311.50±7.23^{c}	1362.50±1.56^{b}	0.000*	0.000*	0.53
35 days	1893.00±2.65^{b}	1979.75±3.75^{a}	1787.50±3.57^{c}	1878.75±6.24^{b}	0.000*	0.000*	0.61
BWG (g)							
Days 1- 7	97.83±1.45	98.63±2.24	93.00±1.58	99.50±1.173	0.26	0.048*	0.11
Days 8- 14	246.91±2.13^{c}	286.63±3.56^{a}	237.63±1.95^{c}	261.00±2.62^{b}	0.000*	0.000*	0.009*
Days 15- 21	379.63±0.66^{b}	396.50±2.96^{a}	339.88±0.47^{c}	344.13±5.38^{c}	0.000*	0.005*	0.06
Days 22- 28	602.50±4.62	601.00±3.32	600.00±8.00	616.88±2.86	0.22	0.16	0.10
Days 29- 35	524.63±5.51^{b}	555.00±2.91^{a}	476.00±4.56^{c}	516.25±6.14^{b}	0.000*	0.000*	0.34
Days 1-35	1851.50±2.65^{b}	1937.75±3.75^{a}	1746.50±3.57^{c}	1837.75±6.24^{b}	0.000*	0.000*	0.57

^{a,b,c,d} Means within a row with different superscripts significantly differ (Tukey's test; P≤0.05).
NT (control): Normal temperature without dietary PFA; NT+PFA: Normal temperature with 1 kg/ton feed dietary PFA"Herb-ALL™ RESP"; LT: Low temperature without dietary PFA; LT+PFA: Low temperature with 1 kg/ton feed dietary PFA"Herb-ALL™ RESP" .
BW: Body weight; BWG: Body weight gain.
Number of sampled birds (n) =30 birds/replicate (N=120 birds/group).

Table 3. Weekly feed intake, Feed conversion ratio, European production efficiency factors, and mortality of broiler chickens supplemented with PFA under low temperature conditions.

Parameters	Treatments						
	NT	NT+PFA	LT	LT+PFA	P-value Temperature (T)	P-value Additive (A)	P-value Interaction (T x A)
WFI (g)							
Days 1- 7	108.25±2.31^{b}	109.50±1.21^{b}	128.50±1.02^{a}	128.00±1.08^{a}	0.000*	0.81	0.57
Days 8- 14	354.38±1.25^{c}	375.71±2.16^{b}	420.88±1.63^{a}	370.86±1.09^{b}	0.000*	0.000*	0.000*
Days 15- 21	622.13±1.31^{a}	625.50±1.85^{a}	626.50±1.71^{a}	592.50±1.62^{b}	0.000*	0.000*	0.000*
Days 22- 28	979.75±2.59^{a}	931.25±1.31^{c}	970. 00±1.62^{a}	955.00±3.19^{b}	0.010*	0.000*	0.000*
Days 29- 35	1095.38±5.18^{c}	1137.25±2.39^{b}	1217.63±4.02^{a}	1205.13±5.11^{a}	0.000*	0.005*	0.000*
Days 1-35	3159.88±1.83^{c}	3179.21±4.69^{c}	3363.50±6.69^{a}	3251.50±5.73^{b}	0.000*	0.000*	0.000*
FCR (feed/gain)							
Days 1- 7	1.11±0.02^{b}	1.11±0.02^{b}	1.38±0.03^{a}	1.29±0.02^{a}	0.000*	0.09	0.06
Days 8- 14	1.44±0.02^{b}	1.31±0.01^{c}	1.77±0.01^{a}	1.42±0.01^{b}	0.000*	0.000*	0.000*
Days 15- 21	1.64±0.00^{c}	1.58±0.01^{c}	1.84±0.00^{a}	1.72±0.02^{b}	0.000*	0.000*	0.07
Days 22- 28	1.62±0.02^{a}	1.55±0.01^{b}	1.62±0.02^{a}	1.55±0.02^{b}	0.73	0.000*	0.80
Days 29- 35	2.09±0.03^{c}	2.05±0.01^{c}	2.56±0.03^{a}	2.34±0.02^{b}	0.000*	0.000*	0.003*
Days 1-35	1.71±0.00^{c}	1.64±0.00^{d}	1.93±0.00^{a}	1.77±0.00^{b}	0.000*	0.000*	0.000*
EPEF	306.40±6.93^{b}	338.99±2.45^{a}	229.83±5.14^{c}	283.21±6.65^{b}	0.000*	0.000*	0.09
Mortality%	3.33±1.92^{c}	1.67±0.96^{c}	13.33±1.93^{ab}	6.67±1.92^{bc}	0.001*	0.033*	0.18

^{a,b,c,d} Means within a row with different superscripts significantly differ (Tukey's test; P≤0.05).
NT (control): Normal temperature without dietary PFA; NT+PFA: Normal temperature with 1 kg/ton feed dietary PFA"Herb-ALL™ RESP"; LT: Low temperature without dietary PFA; LT+PFA: Low temperature with 1 kg/ton feed dietary PFA"Herb-ALL™ RESP".
WFI: Weekly feed intake; FCR, Feed Conversion Ratio (g of feed/g of weight gain); EPEF: European production efficiency factor.
Number of sampled birds (n)=30 birds/replicate (N=120 birds/group).

formance parameters in our experimental studies. Concerning the normal temperature (NT) conditions, there was a significant improvement ($p<0.05$) in the BW in the NT+PFA group from day 14, till 35 of age, in the BWG at days 14, 21, 35 and the overall period, in the FCR at days 14, 28, and the cumulative period, and the EPEF compared to the NT group. However, no significant differences were observed between the two groups in cumulative feed intake or mortality rate.

Regarding the low temperature (LT) conditions, the dietary PFA supplementation in the LT+PFA group showed a significant ($p<0.05$) higher live BW from day 7 till 35 of age, a higher BWG at days 14, 35 and the overall period, decrease in the FCR from day 14 till 35 of age and the cumulative period, and enhancement in the EPEF compared to the LT group. Meanwhile, the LT+PFA group showed a significant reduction ($p<0.05$) in the WFI from day 14 till 28 and the overall period, with a non-significant decrease in the mortality rate compared to the LT group.

ND vaccine's immune status

The mean haemagglutination inhibition (HI) antibody titers against Newcastle Disease Virus (NDV) were evaluated across four groups (Fig. 1). The NT+PFA group had the highest mean HI titer, measuring 8.33 ± 1.20 log2, followed by the NT group with a titer of 7.33 ± 0.33 log2. The LT+PFA group had a mean titer of 4.67 ± 0.33 log2, while the LT group had the lowest titer at 3.67 ± 0.67 log2. The results showed statistically significant higher titers in the NT and NT+PFA groups compared to the LT and LT+PFA groups ($p = 0.006$). These findings indicate variability in the immune response to NDV vaccination across the groups, with NT+PFA demonstrating the strongest antibody response.

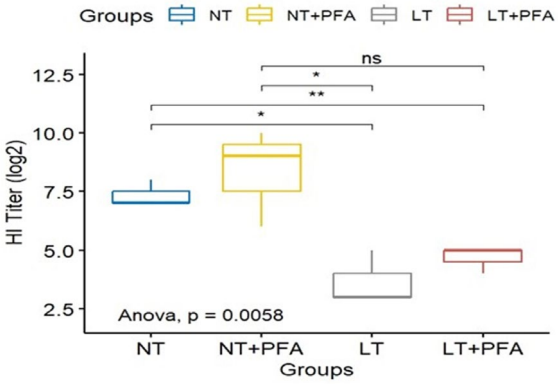

Fig. 1. Box plot showing NDV haemagglutination inhibition (HI) antibody titers among the four bird groups. NT (control): Normal temperature without dietary PFA; NT+PFA: Normal temperature with 1 kg/ton feed dietary PFA; LT: Low temperature without dietary PFA; LT+PFA: Low temperature with 1 kg/ton feed dietary PFA. Asterisks indicate levels of significance: $p < 0.05$ (*), $p < 0.01$ (**), not significant $p > 0.05$ (ns).

Tracheal and lung NRF-2/ IL-1β gene expression

In both the lung and tracheal tissue, we recorded that the *NRF-2* gene is upregulated in PFA groups, indicating the progress of antioxidant status in the lung tissue due to PFA supplementation. The LT group showed downregulation of the *NRF-2* transcription, which is modulated by PFA, leading to significant upregulation of the *NRF-2* gene in the LT+PFA group (Fig 2). In contrast, the LT group showed upregulation of the *IL-1β* due to the cold stress. However, the *IL-1β* mRNA level was downregulated in the PFA-treated groups (Fig 3).

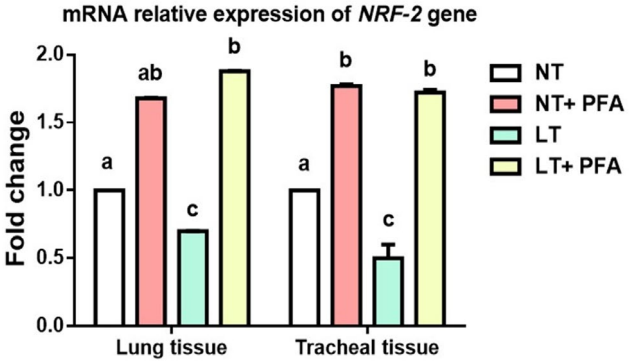

Fig. 2. Lung and tracheal *NRF-2* gene expression among the four bird groups. NT (control): Normal temperature without dietary PFA; NT+PFA: Normal temperature with 1 kg/ton feed dietary PFA; LT: Low temperature without dietary PFA; LT+PFA: Low temperature with 1 kg/ton feed dietary PFA. [a,b,c] different superscript letters indicate a significant difference ($p<0.05$).

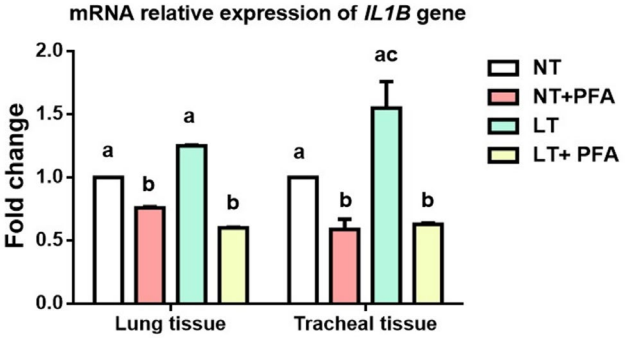

Fig. 3. Lung and tracheal *IL-1β* gene expression among the four bird groups. NT (control): Normal temperature without dietary PFA; NT+PFA: Normal temperature with 1 kg/ton feed dietary PFA; LT: Low temperature without dietary PFA; LT+PFA: Low temperature with 1 kg/ton feed dietary PFA. [a,b,c] different superscript letters indicate a significant difference ($p<0.05$).

Discussion

Cold stress can negatively impact broiler growth performance, including BW and feed efficiency (Li *et al*., 2020; Zhou *et al*., 2021). In this study, cold stress reduced broiler BW, BWG, and EPEF while increasing FI, FCR, and mortality. However, dietary PFA with *Cinnamomum cassia* and *Emblica officinalis* restoring growth performance. Cold stress reduces growth performance mainly because it raises energy metabolism and basal metabolic rate, forcing animals to use more energy to maintain body temperature (Gong *et al*., 2023). This causes an increase in FI and a redirection of nutrients from growth promotion to thermoregulation, resulting in a decrease in BW and BWG and an increase in FCR of broilers. Moreover, cold stress activates the hypothalamus-pituitary-adrenocortical response axis, resulting in elevated levels of ACTH and CORT (Fu *et al*., 2022). This hormonal surge is a stress-adapted mechanism to manage energy homeostasis, but it has downstream consequences for immune response and growth performance (Olfati *et al*., 2018). Additionally, cold stress in broilers disrupts the balance between lipid peroxidation and antioxidant defenses, resulting in excess free radicals and oxidative damage (Gao *et al*., 2025). This was evidenced by decreased mRNA expression of the antioxidant gene *NRF-2* and increased pro-inflammatory cytokine *IL-1β* expression in trachea and lungs, ultimately contributing to reduction of growth performance.

Incorporating particular feed additives and reformulating broiler diets are crucial strategies to maintain production efficiency (Abdelli *et al*., 2021). In recent years, PFAs derived from herbs, spices, and essential oils have gained significant attention as natural alternatives to antibiotic growth promoters (Murugesan *et al*., 2015). In this study, adding *Cinnamomum cassia* and *Emblica officinalis* to broiler diets enhanced growth and production efficiency. Previous studies have shown that using *Cinnamomum cassia* and *Emblica officinalis* feed additives can improve broiler performance in natural (Chavan, 2016; Patel *et al*., 2016)

and stressed environmental conditions (Kazal *et al*., 2023; Khan *et al*., 2023). The improved growth performance is likely due to the supplements enhancing digestive secretions, nutrient absorption, and energy metabolism, leading to better feed utilization (Kumar *et al*., 2014; Dalal *et al*., 2018). Additionally, the immune stimulating effects of *Cinnamomum cassia* and *Emblica officinalis* increase the HI titers and immune status of broilers (Kaleem *et al*., 2014; Meteab *et al*., 2022), therefore ameliorating the hypothalamus-pituitary-adrenocortical axis activation, mitigating stress responses, and enhancing the growth performance (Torki *et al*., 2015; Selvam *et al*., 2018). Moreover, the anti-inflammatory and antioxidant effects of *Cinnamomum cassia* and *Emblica officinalis* observed by the upregulation of tracheal and lung *NRF-2* and downregulation of *IL-1β* gene expression (Lee *et al*., 2023; Liu *et al*., 2024), may improve growth performance by reducing oxidative stress and inflammation brought on by cold stress.

The antioxidant, anti-inflammatory, and immunostimulant properties of *Cinnamomum cassia* are likely due to its high levels of polyphenols and bioactive compounds, particularly Cinnamaldehyde, along with phenolic acids such as Cinnamic, Gallic, Caffeic, and Ferulic acids, and flavonoids including Kaempferol, Rutin, and Quercetin (Al-Numair *et al*., 2007; Klejdus and Kováčik, 2016; Davoudi and Ramazani, 2024). While of *Emblica officinalis* are due to high content of Vitamin C (Ascorbic Acid), Tannins (Emblicanin A and B, Punigluconin and Pedunculagin), Phenolic acid (Gallic acid, Chlorogenic acid, and Ellagic acid), and Flavonoid (Myricetin, Rutin, Kaempferol, and Quercetin) (Shinde *et al*., 2007; Prananda *et al*., 2023).

Regarding the immune status of broiler chickens, broilers under cold stress (LT) had the lowest HI titers, aligning with findings that thermal stress weakens poultry immune function by disrupting lymphoid organ activity and antibody production (Olfati *et al*., 2018). Conversely, the LT+PFA group showed improved HI titers compared to the cold-stressed unsupplemented group, indicating the potential of the PFA to offer partial immunoprotection, likely due to the properties of *Cinnamomum cassia* and *Emblica officinalis* in enhancing antibody responses and reducing inflammation (Singh *et al*., 2014; Lee *et al*., 2023). Notably, the NT+PFA group had the highest HI titers, exceeding both the unsupplemented thermoneutral controls (NT) and all cold-stressed groups, suggesting that phytogenic supplementation boosts humoral immunity in optimal conditions by promoting gut health and immune cell function (Tomar *et al*., 2018; Metea *et al*., 2022).

In relation to tracheal and lung gene expression, *NRF-2* is an essential transcription factor that controls genes expression responsible for antioxidant and cytoprotective functions (Ali *et al*., 2022). Its main function is to protect cells from oxidative stress by activating genes that detoxify reactive oxygen species (ROS) and maintain cellular redox balance (Abd-Eltawab *et al*., 2022). The upregulation of *NRF-2* in lung and tracheal tissues of PFA groups indicates that PFA may boost antioxidant defense mechanisms through NFF-2 activation. Conversely, the cold stress impairs the *NRF-2* expression, potentially reducing the chicks' ability to respond to the oxidative stress. However, the PFA resulted in a significant increase in *NRF-2* expression in the LT group, suggesting that the PFA may mitigate some of the cold stress adverse effects by promoting *NRF-2*-mediated antioxidant responses. Cold stress increases oxidative stress due to metabolic changes and higher ROS production. Assessing *NRF-2* expression reveals the body's capacity to counteract oxidative damage (Blagojevic *et al*., 2011). Increased *NRF-2* expression indicates a robust antioxidant response, essential for protecting cells under stress and regulating inflammation, especially in sensitive tissues like the lungs and trachea (Vomund *et al*., 2017).

IL-1β is a pro-inflammatory cytokine produced by immune cells in response to infection or tissue damage, playing a key role in initiating and sustaining inflammatory response (Lopez-Castejon and Brough, 2011). The *IL-1β* expression also showed notable differences across the experimental groups. The PFA induced downregulation of the *IL-1β* levels in the lung and the trachea, indicating a potential anti-inflammatory effect of the PFA in the NT+PFA group. In contrast, the cold stress groups exhibited upregulation of *IL-1β* in both the lung and trachea. This suggests that cold stress may provoke an inflammatory response, leading to upregulation of the *IL-1β* expression. However, the PFA supplementation in the LT + PFA group significantly reduced *IL-1β* transcritption. Cold stress can trigger the production of *IL-1β*, resulting in increased inflammation. Higher levels of *IL-1β* can signal tissue damage and the intensity of the inflammatory response (Mendiola and Cardona, 2018). Analyzing *IL-1β* expression aids in assessing the degree of cold-induced injury, especially in vulnerable tissues. Additionally, *IL-1β* plays a role in recruiting and activating other immune cells (Vasek *et al*., 2024).

Conclusion

Cold stress negatively impacted broiler performance, immune response, and respiratory antioxidant and inflammatory gene expression. Supplementation with the PFA (Herb-ALL™ RESP), containing *Cinnamomum cassia* and *Emblica officinalis*, effectively alleviated these adverse effects by improving growth performance, enhancing immune response against NDV, upregulating the antioxidant *NRF-2* gene, and downregulating the pro-inflammatory *IL-1β* gene in lung and tracheal tissues. These findings support the use of PFA as a practical nutritional intervention to safeguard broiler health and performance under cold environmental stress.

Conflict of interest

The authors have no conflict of interest to declare.

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