RESEARCH ARTICLE

Synergistic Effect of Cinnamon Oil and Butyric Acid on Growth Performance, Oxidative Stress, Immunological and Intestinal Histomorphology of Broiler Chickens

Raina Doneria[1]*, Meenu Dubey[1], Manoj Kumar Gendley[1], Rupal Pathak[2], Shraddha Nety[3], Mehtab Singh Parmar[4]

Abstract

The present experiment evaluated the effect of cinnamon oil (CEO) and butyric acid (BA) on the performance, oxidative stress parameters, immune response and intestinal histomorphology of broiler chickens. Day-old broiler chicks (n=150) were distributed randomly into five experimental groups. The experimental groups were control: T0 (basal diet without bacitracin), T1 (basal diet + bacitracin @ 50 mg/kg diet), T2 (basal diet + 400 mg/kg CEO), T3 (basal diet + 0.4 % BA), T4 (basal diet + 400 mg/kg CEO + 0.4% BA). Our results revealed that feed intake was significantly lower in T4, though a significant escalation in overall weight gain, and feed conversion ratio (FCR) was observed. Furthermore, T4 group exhibited a significantly higher defeathered, eviscerated, dressed weight and increased glutathione concentration as compared to T0. The haemagglutination inhibition (HI) titer against Newcastle Disease Virus (NCDV) was significantly higher in all the treatment groups as compared to T0. Additionally, significantly ($p<0.05$) improved villi height of duodenum and jejunum were observed in T4 group as compared to other groups. Conclusively, combining CEO and BA complemented the positive effects of each other and might be suitable substitutes to antibiotics for enhanced performance, immunity and antibiotic residue free meat production in poultry.

Keywords: Broiler, acid, Cinnamon oil, Growth performance, Immune response. Oxidative stress.

Ind J Vet Sci and Biotech (2025)**:** 10.48165/ijvsbt.21.3.14

Introduction

In the modern era, mounting pressure to trim down the use of antimicrobial growth promoters (AGPs) in food animals makes it crucial to find alternatives that provide consumers with safe food without compromising the productivity of the animals. Essential oils (EOs) present in phytogenic feed additives (PFA) and organic acids (OAs) are some of these optional strategies which are likely to offer prospective benefits during the post-AGP epoch. EOs and OAs collectively may pave the way for meat producers to produce antibiotic residue free meat. In India, cinnamon (*Cinnamomum zeylanicum*) is among an ancient medicinal plant, extensively used as condiment. Myriad studies conducted with broiler chickens and poultry diets supplemented with cinnamon and their EOs, anti-microbial activities against various microorganisms and potent antioxidant activities have been recorded (Ali *et al.,* 2021). Similarly, short chain fatty acids are a budding substitutes to antibiotic growth promoter (Van Immerseel *et al.,* 2003). Short chain fatty acid like butyric acid have bactericidal activity in un-dissociated acid form (Leeson, 2005). In addition, butyric acid, decreases pathogenic bacteria and their toxins, improves gut health, in doing so escalates nutrient digestibility and consequently leads to enhanced growth performance and immunity among birds (El-Saadony *et al.,* 2022).

Thus, investigating the potential synergistic or amalgamated benefits of EOs with other feed additives like OAs is of far more significance than exploring the individual components (Yang *et al.,* 2018). Reports are extremely scanty, where dietary supplementation of these two key players, *i.e.,* CEO and BA have been used. Thus to add new insights to the existing knowledge, the present study was undertaken to investigate the effect of dietary supplementation of CEO and BA individually and in combination on growth performance, oxidative stress parameters, immune response and intestinal histomorphology of broilers and to further assess their role as an alternative growth promoter in poultry feeds.

[1]Department of Animal Nutrition, College of Veterinary Science & AH, Dau Shri Vasudev Chandrakar Kamdhenu Vishwavidyalaya (DSVCKV), Anjora, Durg-491001, Chhattisgarh, India

[2]Department of Livestock Production Management, College of Veterinary Science & AH, DSVCKV, Anjora, Durg-491001, Chhattisgarh, India

[3]Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science & AH, DSVCKV, Anjora, Durg-491001, Chhattisgarh, India

[4]Department of Veterinary Physiology & Biochemistry, College of Veterinary Science & AH, DSVCKV, Anjora, Durg-491001, Chhattisgarh, India

***Corresponding author:** Dr. Raina Doneria, Department of Animal Nutrition, College of Veterinary Science and Animal Husbandry, DSVCKV, Anjora, Durg-491001, Chhattisgarh, India. E-mail: raina.doneria@gmail.com

How to cite this article: Doneria, R., Dubey, M., Gendley, M. K., Pathak, R., Nety, S., & Parmar, M. S. (2025). Synergistic Effect of Cinnamon Oil and Butyric Acid on Growth Performance, Oxidative Stress, Immunological and Intestinal Histomorphology of Broiler Chickens. Ind J Vet Sci and Biotech, 21(3), 68-72.

Source of support: Nil

Conflict of interest: None

Submitted 29/01/2025 **Accepted** 01/03/2025 **Published** 10/05/2025

© The Author(s). 2025 Open Access This work is licensed under a Creative Commons Attribution-Non Commercial-No Derivatives 4.0 International License.

Materials and Methods

Birds and Experimental Diet

Ross AP strain one-day old broiler chicks (n=150) were reared under deep litter system for 35 days. The chicks were divided into 5 groups of three replicates with dietary treatment groups (T0-T4). The diets were formulated as per ICAR, 2013 using maize, soy DOC, soy oil along with minerals and vitamin premixes. Five types of isocaloric and isonitrogenous diets were formulated. Diet 1 was basal diet without AGP served as negative control (T0), diet 2 consisted of basal diet with AGP bacitracin @ 50 mg/kg diet (T1), diets 3: basal diet + 400 mg/kg CEO (T2), diet 4: basal diet+ 0.4% BA (T3) and diet 5: basal diet + 400 mg/kg CEO and 0.4% BA in combination (T4). The inclusion levels of CEO @ 400 mg/kg and BA @ 0.4% were deduced from the previous findings from our laboratory (Doneria *et al.,* 2022[a,b]). The cinnamon bark oil and butyric acid used for the dietary supplementation in this study were purchased from Allin Exporters, Noida, India and Loba Chemie, Mumbai, India, respectively.

Performance Study

The feed intake on daily basis and weekly body weight were recorded to reach at overall body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR). In order to study the carcass characteristics, three birds from each replicate were slaughtered on 35th day of the experiment.

Oxidative Stress Indices and Immune Response

To evaluate the oxidative stress the lipid peroxidation was analysed as per the method of Placer *et al.* (1966) and Reduced Glutathione in RBC suspension as per the method suggested by Prins and Loos (1969). The *in vivo* cell mediated immune response to phytohaemaglutinin P (PHA-P) was analysed by the previously described method (Cheng and Lamont, 1988). The mean serum haemagglutination inhibition (HI) titre was estimated against NCDV as per the method of Allan and Gough (1974).

Histological Examination

The representative samples of duodenum, jejunum and liver were processed and stained as per the procedure described by Singh and Sulochana (1997). The height of the villi and depth of crypt were measured using an inverted microscope (T capture software).

Statistical Analyses

The data were analyzed by one-way Analysis of Variance (Snedecor and Cochran, 1989) and mean values were compared by Duncan multiple range test. The mean values were compared using SPSS package (SPSS ver 26.0) and the significance difference was expressed at 1 and 5 % of probability.

Results and Discussion

Growth Performance

The growth performance due to dietary supplementation was significantly ($p<0.05$) higher in birds fed CEO+BA supplemented diet as compared to other groups (Table 1). The present findings aligned with previous studies in which greater gain and feed efficiency were reported in broilers fed blends of essential oils and organic acids compared to control diet (Malayoğlu *et al.,* 2016; Gao *et al.,* 2019). Correspondingly, some researchers found that blend of encapsulated essential oils and organic acids supplementation improved the growth performance in challenged broiler chickens (Stefanello *et al.,* 2020; Pham *et al.,* 2021). However in contrary, some studies concluded that dietary supplementation of blend of essential oil and organic acid has no effect on the growth performance of broiler chicken (Isabel and Santos, 2009).

The birds of group T4 supplemented with CEO+BA in combination consumed significantly less feed as compared to other groups. The findings were in accordance with Chowdhury *et al*. (2021). Conversely, many researchers found no difference in feed consumed throughout the experiment (Pathak *et al.,* 2016; Yang *et al.,* 2018). In our study, significantly enhanced FCR was observed in birds of T4 group supplemented a blend of CEO + BA than other groups. The findings were in accordance with various workers (Isabel and Santos, 2009; Malayoğlu *et al.,* 2016; Yang *et al.,* 2018; Chowdhury *et al.,* 2021) who reported improved FCR in essential oil and organic acid combine supplemented group. In contrast, no effect on growth performance by the addition of commercial blend of cinnamaldehyde and thymol or its combination with Na-butyrate in feed was observed by Cerisuelo *et al.* (2014).

Table 1: Effect of cinnamon oil and butyric acid alone or in combination on growth performance of broilers (0-35 d)

Particulars	Dietary treatment groups					P value
	T0	T1	T2	T3	T4	
Feed intake (g)	3437.06^{a}±89.99	3263.50^{ab}±90.34	3019.70^{c}±28.91	3163.16^{bc}±12.99	2920.13^{d}±73.00	0.01
B.wt. gain (g)	1717.19^{d}±75.82	1842.56^{c}±12.55	1975.32^{b}±20.32	1903.15^{bc}±22.20	2026.33^{a}±11.21	0.01
FCR	2.00^{a} ±0.05	1.77^{b} ±0.03	1.53^{c} ±0.02	1.66^{c} ±0.04	1.44^{d} ±0.01	0.01

Means in the same row with different superscript a, b, c differ significantly at $p<0.05$.

Oxidative Stress Indices and Immune Response

The oxidative stress level and immune response are presented in Table 2. No significant effect of dietary supplementation on melondialdehyde concentration was observed among the groups. However, we observed a significantly higher glutathione concentration in birds of supplemented groups as compared to T0 group. This might be ascribed to the phenolic compounds of cinnamon oil which possess the ability to scavenge the free radicals and play a significant protecting role against tissue damage and inflammation (Alves-Santos *et al.,* 2020). Furthermore, the HI titer against NCDV on 28th and 35th day was found significantly higher in T2, T3 and T4 as compared to T0. Our results are in line with earlier reports where higher antibody titer against NCDV in EO + OA supplemented group was registered than in NC, AGP and OA groups (Fascina *et al.,* 2012; Pathak *et al.,* 2016).

Table 2: Effect of cinnamon oil and butyric acid alone or in combination on oxidative stress indices, cell mediated and humoral immune response in broilers

Particulars	Dietary treatment groups					P value
	T0	T1	T2	T3	T4	
Lipid peroxidation (MDA in nM/mg Hb)	4.02±0.07	3.86±0.33	3.64±0.75	3.62±0.58	3.18±0.83	0.876
GSH (M/g Hb)	0.22^{b}±0.02	0.26^{ab}±0.01	0.29^{a}±0.02	0.27^{a}±0.01	0.29^{a}±0.01	0.01
FI (mm) after 24 h	0.46±0.03	0.58±0.01	0.65±0.02	0.64±0.06	0.65±0.05	0.06
HI titer (log 2) at 28 d	2.33^{b}±0.33	3.33^{ab}±0.67	4.32^{a}±0.33	4.33^{a}±0.33	4.69^{a}±0.33	0.01
HI titer (log 2) at 35 d	2.00^{b}±0.58	3.67^{a}±0.67	4.01^{a}±0.58	4.66^{a}±0.33	4.00^{a}±0.58	0.02

Means in the same row with different superscript a, b, c differ significantly at p<0.05

Carcass Traits

The effects of different dietary supplementation on the carcass characteristics are presented in Table 3. Statistical analysis revealed significantly (p<0.05) increased defeathered weight, eviscerated weight, dressing percentage in CEO + BA supplemented T4 group as compared to CEO,BA and NC groups (T2,T3,T0). The results of the carcass traits are in general agreement with researches who reported marked increase in carcass yield specially eviscerated weight in essential oil and organic acid group (Iqbal *et al.,* 2021). However, contrary to our findings, few studies revealed no effect on carcass weight and relative organ weights of broilers by the supplementation of essential oil and organic acid blend (Gomathi *et al.,* 2018).

Table 3: Effect of cinnamon oil and butyric acid alone or in combination on carcass traits (% live wt) of broilers at 35 d

Particulars (%live wt)	Dietary treatment groups					p value
	T0	T1	T2	T3	T4	
Defeathered wt	71.17^{b}±1.12	77.35^{a}±0.38	70.87^{b}±0.39	70.4^{b}±0.29	78.95^{a}±0.97	0.01
Eviscerated wt	56.95^{c}±2.00	68.15^{ab}±1.1	62.11^{bc}±1.58	59.74^{c}±2.73	72.35^{a}±1.91	0.01
Dressed wt	62.30^{b}±1.39	74.14^{a}±.90	65.56^{b}±3.66	64.92^{b}±.85	78.09^{a}±.85	0.01

Means in the same row with different superscript a, b, c differ significantly at p<0.05.

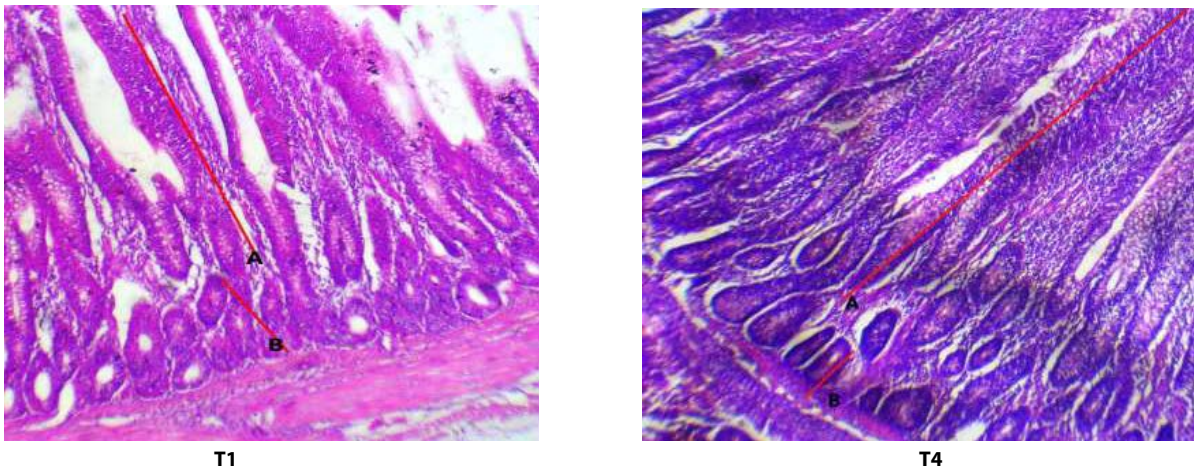
Intestinal Histology

The data indicated significant improvement in villus height of duodenum and jejunum in birds fed diet supplemented with CEO + BA combination (T4) as compared to other groups (Table 4, Fig. 1). No significant effect of different dietary supplementation of CEO and BA alone or in combination on crypt depth and VH: CD ratio of jejunum was observed in the present study. These findings were in consistent with the results of previous studies (Malayoğlu *et al.,* 2016; Gao *et al.,* 2019; Zhang *et al.,* 2019; Pham *et al.,* 2021), who reported increased intestinal morphometry in essential oil and organic acid blend as compared to control group. However, some previous reports indicated no interaction between EO and OA on small intestine histology and no significant effect on the crypt depth and villi surface area (Phuengkasem *et al.,* 2017). Furthermore, improved intestinal morphology may be due to the anti-inflammatory and anti-oxidation mechanism of essential oils and organic acids (Gao *et al.,* 2019). Villus height of intestine, crypt depth and their ratio not only influences the function of intestinal digestion and absorption but also relate to the colonization of harmful bacteria (Zhang *et al.,* 2019). Additionally, in the present study no pathological changes were observed in the histological section of liver indicating no adverse effect of dietary supplementation of essential oil and organic acid alone or their combinations in broiler chicken.

Table 4: Effect of cinnamon oil and butyric acid alone or in combination on intestinal histo-morphology of broilers at 35 d of age

Particular		Dietary treatment groups					P value
		T0	T1	T2	T3	T4	
Duodenum	VH (µm)	1115^{b}±55.41	1201^{b}±36.59	1222^{b}±40.10	1213.33^{b}±6.69	1423^{a}±27.71	0.01
	CD (µm)	229.66^{b}±10.87	232.67^{b}±16.71	247^{b}±23.07	206.33^{b}±32.42	313.33^{a}±20.53	0.01
	VH: CD	4.86±0.29	5.20±0.69	5.01±0.26	5.92±0.66	4.70±0.44	0.13
Jejunum	VH (µm)	1179^{b}±17.43	1206^{b}±20.13	1220^{b}±93.24	1271^{b}±87.68	1483.67^{a}±49.1	0.01
	CD (µm)	210±14.73	251.33±26.03	274.33±9.39	274.67±31.85	253.67±11.29	0.24
	VH: CD	5.61±0.33	4.89±0.67	4.44±0.33	4.75±0.32	5.84±0.12	0.01

VH= villi height, CD= crypt depth. Means in the same row with different superscript a, b, c differ significantly at p<0.05.



T1 T4

Fig. 1: Photomicrograph of duodenum of birds under treatment T1 and T4 showing height of villi (A), crypt depth (B) (H & E, 10X)

Conclusion

The study revealed that the blend of CEO @ 400 mg/kg and BA @ 0.4% complemented the positive effects of each other and was found more effective than the individual additive as reflected in performance, intestinal histology and immune response. The application of cinnamon oil and butyric acid in combination can be used as a potential approach to create a new and safe feed additive for broilers.

Acknowledgement

The authors express their sincere gratitude to Dau Shri Vasudev Chandrakar Kamdhenu Vishwavidyalaya, Chhattisgarh, India for providing necessary facilities and fund for the present experiment.

References

Ali, A., Ponnampalam, E.N., Pushpakumara, G., Cottrell, J.J., Suleria, H.A.R., & Dunshea, F.R. (2021). Cinnamon: A natural feed additive for poultry health and production-A review. *Animals* (Basel), *7,* 2026

Allan, W.H., & Gough, R.E. (1974). A standard HI test for NCDV I. Comparison of macro and micro method. *Veterinary Record, 95,* 120-125.

Alves-Santos, A.M., Sugizaki, C.S.A., Lima, G.C., & Naves, M.M.V. (2020). Prebiotic effect of dietary polyphenols: A systematic review. *Journal of Functional Foods, 74,* 104169.

Cerisuelo, A., Marin, C., Sanchez-Vizcaino, F.. Gomez, E.A., De La Fuente, J.M., Duran, R., & Fernandez (2014). The impact of a specific blend of essential oil components and sodium butyrate in feed on growth performance and Salmonella counts in experimentally challenged broilers. *Poultry Science, 93,* 599-606.

Cheng, S., & Lamont, S.J. (1988). Breeding and genetics, genetic analysis of immune competence in White Leghorn chicken line. *Poultry Science, 67,* 989-995.

Chowdhury, M.A.K., Dhara, A.K., Dey, S., Sarkar, A., Tactacan, G., & Haldar S. (2021). Dietary supplementation of a microencapsulated essential oil-organic acid blend with and without a protease affects performance, nutrient accretion, cecal microbiota, and circulatory biomarker concentrations in male broiler chickens. *Letters to Animal Biotechnology, 1*, 19-32.

Doneria, R., Dubey, M., Gendley, M.K., Chourasia, D., Pathak, R., Ramteke,R.C., Prusty,S., & Parmar, M.S. (2022[a]). Impact of dietary supplementation of cinnamon oil on the oxidative stress indices, immune response and intestinal morphology in broiler chickens. *Indian Journal of Animal Sciences, 92,*991-994.

Doneria, R., Dubey, M., Gendley, M.K., Pathak, R., Parmar, M.S., Dinani, O.P., & Ramteke, R.C. (2022[b]). Impact of dietary supplementation of graded levels of butyric acid on growth

performance and nutrient utilization in broiler chicken. *Indian Journal of Animal Nutrition, 39,* 190-199.

El-Saadony, M.T., Yaqoob, M.U., Hassan, F.U., Alagawany, M., Arif, M., Taha, A.E.m Elnesr, S.S., El-Tarabily, K.A., & El-Hack, M.E.A. (2022). Applications of butyric acid in poultry production: the dynamics of gut health, performance, nutrient utilization, egg quality, and osteoporosis. *Animal Health Research Reviews, 23,* 136-146.

Fascina, V.B., Sartori, J.R., Gonzales, E., De Carvalho, F.B., De Souza, I.M.G.P.V., Polycarpo, G. D., Stradiotti, A.C., & Pelícia V.C. (2012). Phytogenic additives and organic acids in broiler chicken diets. *Revista Brasileira de Zootecnia, 41,* 2189-2197.

Gao, Y.Y., Zhang, X.L., Xu, L.H., Peng, H., Wang, C.K., & Bi, Y.Z. (2019). Encapsulated blends of essential oils and organic acids improved performance, intestinal morphology, cecal microflora, and jejunal enzyme activity of broilers. *Czech Journal Animal Science, 64,* 189-198.

Gomathi, G., Senthilkumar, S., Natarajan, A., Amutha, R., & Purushothaman, M.R. (2018). Effect of dietary supplementation of cinnamon oil and sodium butyrate on carcass characteristics and meat quality of broiler chicken. *Veterinary World, 11,* 959-964.

ICAR (2013). Nutrient specification for broilers. In: *Nutrient Requirements of Poultry*. New India Publication, New Delhi, India, pp. 15.

Iqbal, H., Rahman, A., Khanum, S., Arshad, M., Badar, I.H., Asif, A., Hayat, Z., & Iqbal, M.A. (2021). Effect of essential oil and organic acid on performance, gut health, bacterial count and serological parameters in broiler. *Brazilian Journal of Poultry Science, 23*, 1-10.

Isabel, B., & Santos,Y. (2009). Effects of dietary organic acids and essential oils on growth performance and carcass characteristics of broiler chickens. *Journal of Applied Poultry Research, 18,* 472-476.

Leeson S., Namkung H., Antongiovanni, M., & Lee. E. H (2005). Effect of butyric acid on the performance and carcass yield of broiler chickens. *Poultry Science, 84,* 1418-1422

Malayoğlu, H.B., Ozdemir, P., & Bağriyanik, H.A. (2016). Influence of an organic acid blend and essential oil blend, individually or in combination, on growth performance, carcass parameters, apparent digestibility, intestinal microflora and intestinal morphology of broilers. *British Poultry Science, 57,* 227-234.

Pathak, M., Mandal, G.P., Patra, A.K., Samanta, I., Pradhan, S., & Haldar, S. (2016). Effects of dietary supplementation of cinnamaldehyde and formic acid on growth performance, intestinal microbiota and immune response in broiler chickens. *Animal Production Science, 57,* 821-827.

Pham, V.H., Abbas,W., Huang, J., He, Q., Zhen, W., Guo,Y., & Wang, Z. (2021). Effect of blending encapsulated essential oils and organic acids as an antibiotic growth promoter alternative on growth performance and intestinal health in broilers with necrotic enteritis. *Poultry Science, 101,* 101563.

Phuengkasem, A., Attamangkune, S., & Ruangpanit, Y. (2017). A comparison of essential oil and organic acid supplementation on the live performance and intestinal morphology of broilers fed a diet containing palm kernel meal. *International Journal Poultry Science, 16,* 196-202.

Placer, Z.A., Cushmann, L.L., & Johnson, B.C (1966). Estimation of the product of lipid peroxidation (malonyl dialdehyde) in biochemical systems. *Analytical Biochemistry, 16,* 359-364.

Prins, H.K., & Loos, J.A. (1969). B*iochemical Methods in Red Cell Genetics*. Academic Press, Academic Press, New York, pp. 127-129.

Singh, U.B., & Sulochana, S. (1997). *A Practical Manual of Histopathological and Histochemical Techniques*. Kothari Publication, Bombay, India, pp. 1-41.

Snedecor, G.W., & Cochran W.G. (1989). *Statistical Methods.* 8th edn., Iowa State University Press, Ames, Iowa, USA.

Stefanello, C., Rosa, D.P., Dalmoro, Y.K., Segatto, A.L., Vieira, M.S., Moraes, M.L., & Santin, E. (2020). Protected blend of organic acids and essential oils improves growth performance, nutrient digestibility, and intestinal health of broiler chickens undergoing an intestinal challenge. *Frontier Veterinary Science, 10,* 491.

Van Immerseel, F., Buck, J., De, F., Pasmans, P., Velge, E., Bottreau, Fievez V., Haesebrouck, F., & Ducatelle, R. (2003). The invasion of *Salmonella enteritidis* in avian intestinal epithelial cells *in vitro* is influenced by short-chain fatty acids. *International Journal of Food Microbiology, 85,* 237-248.

Yang, X., Xin, H., Yang, C., & Yang, X. (2018). Impact of essential oils and organic acids on the growth performance, digestive functions and immunity of broiler chickens. *Animal Nutrition, 4,* 388-393.

Zhang, S., Shen, Y.R., Wu, S., Xiao, Y.Q., He, Q., & Shi, S.R. (2019). The dietary combination of essential oils and organic acids reduces *Salmonella enteritidis* in challenged chicks. *Poultry Science, 98,* 6349-6355.