SSRG International Journal of Veterinary Science
ISSN: 2455 – 0868 /doi:10.14445/24550868/IJVS-V6I3P101

Volume 6 Issue 3, 1-6, Sep-Dec 2020

Effect of Supplementing Probiotic, Organic Acid and Herbal Extract (PhytoGrow) on Performance, Egg Quality and Gut Microbiota in White Leghorn Layers

Bhagwat Vishwanath G*[1], Balamurugan Ellusamy[1], Rangesh Paramesh[1], Devanaboyina Nagalakshmi, Thota Srilatha[2], Savaram Venkata Rama Rao[3]

[1]The Himalaya Drug Company, Makali, Bengaluru 562 162, Karnataka, India.
[2]PVNR Telangana Veterinary University, Rajendranagar, Hyderabad -500030, India
[3]Sri Ramadhoota Poultry Research Farm, Flat No. 202, 2nd Floor, M Square, Street No. 01, Patrikanagar, Madhapur, Hyderabad-500081, India

***Abstract**: Due to the demand from consumers, the antibiotic growth promoters are being replaced with alternatives in poultry diets, which include herbal growth promoters. The present study was undertaken to evaluate the effect of a phytogenic feed additive, PhytoGrow, on production performance parameters, egg quality traits, and gut microbiota in White Leghorn (WL) layer birds. A total of 540 WL layer birds, aged 42 weeks, were equally divided into 3 groups (180 birds/group); group 1 received PhytoGrow, group 2 received a probiotic product (Bacillus subtilis), group 3 received (Sodium butyric acid 30%), and PhytoGrow is a blend of potential herbs - Azadirachta indica, Curcuma longa, Andrographis paniculata, and Cinnamomum zeylanicum. The birds in all the 3 groups were raised on a standard layer diet and concurrently supplemented with the above additives at a mixing ratio of 500 g/ton and fed ad libitum for 12 weeks (42 to 53 weeks of age). Data on egg production, body weight, egg quality traits, and gut microbiota were assessed. The results of the present study revealed that there was no significant difference in performance parameters hen day egg production (HDEP), feed intake, feed intake/egg, feed conversion ratio, body weight, and egg weight) and egg quality parameters (egg density, egg strength, Haugh unit, eggshell weight, and eggshell defects) in WL layers fed different additives tested in the study. However, supplementation of PhytoGrow significantly reduced the count of Escherichia coli and Clostridium perfringens in ileum and cecum compared to those fed the other two additives. PhytoGrow supplementation significantly reduced pathogen count and numerically improved the performance and egg quality traits in WL layers during the mid egg production phase (42-53 weeks of age) compared to those fed probiotic and butyric acid in the diet.*

Keywords: *Feed additive, Gut microbiota, Production performance, PhytoGrow, White Leghorn layer birds.*

I. INTRODUCTION

In poultry farming, gut-related issues are quite common. Antibiotic growth promoters (AGPs) are used routinely to overcome such gut-related issues in broilers and layer birds. However, the addition of AGPs in poultry nutrition has been banned globally to avoid the development of cross-resistance and multiple drug resistance in poultry. Hence, AGP's need to be replaced with safe and efficacious alternatives. Among alternatives, acidifiers, enzymes, probiotics, and phytogenic feed additives (herbal products) are to be considered. Much research has been done to explore the use of phytogenic feed additives (plants or parts of plants, plant extracts, essential oils, and single or a combination of essential oils) as effective substitutes [1],[2]. The availability of a variety of plants helps support the thought of using phytogenic feed additives in animal nutrition [3]. As many studies have supported the use of essential oils, they can be used as alternative growth promoters in poultry nutrition. The phytogenic feed additives have several advantages over commonly used antibiotics as they are regularly used and recognized as safe for human consumption also [4],[5].

Furthermore, antimicrobial, antiviral, antifungal, and antioxidant effects of phytogenic feed additives are well described in *in-vitro* studies [6],[7]. An increasing number of *in-vivo* animal nutrition trials that address the gastrointestinal effects of phytogenic feed additives are available. The findings of Windisch *et al.* delineated that the intestinal microflora, gut morphology, gastric emptying, and activity of endogenous digestive secretions, just as the product performance parameters are impacted following favorable supplementation of phytogenic feed additives in the diets poultry feed [8]. The essential oils are essentially extricated by steam refining from various plant materials [9]. Hence, the concoction creation and grouping of dynamic plant mixes fluctuate enormously subject to their source [10], [11].

Studies that examine about impacts of phytogenic feed additives on egg quality attributes *viz.* egg organization, shell thickness, or Haugh unit rating, are scarce. Performance factors that were overwhelmingly watched incorporate feed consumption, FCR, egg production rate, and egg weight. Most investigations finished up with slight beneficial outcomes or patterns. Anyway, noteworthy outcomes were scarce. Since there are practically boundless prospects concerning dose and blends of spices and essential oils, there is still more exploration is required. A dearth of knowledge and evidence of *in-vivo* experiments clues to complications while analysis of results [12].

Until now, there is significant potential to help gut wellbeing and emphatically impact production performance utilizing phytogenic feed added substance. Nonetheless, the utilization of spices, essential oils, or their dynamic plant mixes in commercial layer feed has been examined uniquely in an inadequate number of *in-vivo* preliminaries.

Aim

This study was conducted to evaluate the effect of PhytoGrow, a phytogenic feed additive, on production performance, egg quality traits, and gut microbiota in White Leghorn (WL) layer birds in comparison to two gut health solutions.

II. MATERIALS AND METHODS

A. PhytoGrow: A phytogenic feed additive

PhytoGrow is a proprietary polyherbal formulation developed by The Himalaya Drug Company (Bengaluru, Karnataka, India). PhytoGrow mainly comprises herbs such as *Azadirachta indica*, *Curcuma longa*, *Andrographis paniculata*, and *Cinnamomum zeylanicum*.

B. Experimental diet preparation

The experimental diet was formulated based on the prevailing nutritional standards for egg-laying chicken (BV 300, Venkateswara Hatchery Pvt. Ltd., Hyderabad, India) (Table 1). The PhytoGrow and two other gut health solutions, *i.e.*, Probiotics (*Bacillus subtilis)* and Butyric acid (Sodium Butyric acid 30%), were mixed in the basal diet at the rate of 500 g/ton.

C. General layer birds husbandry practices

The standard management practices required for WL layers were followed during the course of the experiment. The experimental birds were reared in 4-bird colony cages (18″ × 12″ × 15″) using the stair-step cage system. Each cage had access to two nipple drinkers and feeders fixed. The potable drinking water and experimental diets were provided to the bird's *ad libitum* throughout the study period. The recommended standard WL layer bird’s vaccination schedule was followed.

TABLE 1: Ingredients and Nutrient Composition of WL Layer Basal Diet

Ingredient	Quantity (g/kg)
Maize	644.800
Soybean meal	216.600
Vegetable oil	03.390
Dicalcium phosphate	11.680
Stone grit	115.600
Salt	4.138
DL-methionine	1.393
Trace mineral premix	1.000
Vitamin premix	0.500
Choline chloride	0.500
Nutrient Composition	
ME, kcal/kg	2650.000
Crude protein, %	15.500
D-lysine	0.710
D-TSAA	0.600
D-tryptophan	0.166
D-threonine	0.553
D-isoleucine	0.592
D-valine	0.664
Calcium, %	3.800
NPP, %	0.300
Na	0.170
Abbreviations: ME: Metabolizable Energy; Na: Sodium; NPP: Non-phytate phosphorus	

D. Ethical approval

The present study was conducted according to guidelines laid down for the care and use of animals, and the study protocol was approved by the Institutional Animal Ethics committee, The Himalaya Drug Company, Bangalore, Protocol No. AHP/P/09/17.Institutional Ethics Committee.

E. Study design

A total of 540 WL layer birds aged 42 weeks were allocated equally into 3 groups (9 replicates/group; 20 birds/replicate). The birds in group 1 received PhytoGrow; group 2 received a probiotic product (Min. $1x10^{10}$ cfu/g), and group 3 received coated butyric acid (30%). The birds in all the 3 groups were raised on a standard layer mash feed and concurrently supplemented with the additives each at 500 g/ton. Each diet was fed *ad libitum* to 9 replicates, having 20 egg-laying hens from 42 to 53 weeks of age. The birds were hosed in 4 bird colony cages, and 5 adjacent cages had a common feeding considered as a replicate.

F. Assessment parameters

***a) Production parameters*:** Hen housed egg production (HDEP), feed intake (FI), FI/egg, feed conversion ratio (FCR), body weight (BW), and egg weight (EW) were evaluated on a weekly basis. The reported cumulative production performance results were evaluated to study the effect of the addition of PhytoGrow, Probiotic, and coated butyric acid in WL commercial layers.

***b) Egg quality parameters*:** The egg quality was assessed in terms of egg density, egg strength, Haugh unit, eggshell weight, and eggshell defects. Egg quality was measured by collecting 3 eggs from each replicate during the last 3 days of each 28d period..

***c) Microbial count*:** At the end of study (53 weeks of age), one bird from each replicate was slaughtered by cervical dislocation, and about 1 gram of digesta each from ileum and cecum were collected aseptically and mixed with 9 mL saline to prepare a 10-fold dilution. From each dilution, 1 mL was spread on brilliant green agar plate and incubated at 37°C for 24 h. The total colony count of *Clostridium perfringens* and *Escherichia coli* was calculated. The microbial counts were determined as colony forming units (CFU) per gram of the sample [13].

d) Statistical analysis

The data collected were subjected to one way ANOVA according to a completely randomized design followed by Dunnett's multiple comparison post hoc test [14]. $p<0.05$ was considered statistically significant.

III. RESULTS

The performance parameters such as HDEP, FI, FI/egg, FCR, BW, and EW of WL layer birds were found to be improved nonsignificantly ($p>0.05$) following supplementation of PhytoGrow. However, there is a numerical difference between PG vs. Probiotics and Butyric acid fed layers. The HDEP improved by 0.87% and 0.55% in the PhytoGrow-supplemented group compared with the groups that received Probiotic and Butyric acid, respectively. The FCR values depicted that layer birds consumed 20 and 30 g less feed per unit egg mass gain in the PhytoGrow-supplemented group compared with Probiotic and Butyric acid groups, respectively. The birds supplemented with PhytoGrow gained 24.65 and 4.92 g more BW compared with the birds supplemented with Probiotic and Butyric acid, respectively. Furthermore, EW improved by 0.16 g in the PhytoGrow-supplemented group compared with the Probiotic group (Table 2). However, statistically, all the performance variables in PG fed layers were similar to those fed gut health solutions.

The egg quality parameters such as egg density, egg strength, Haugh unit, eggshell weight, and eggshell defects of WL layer birds were found to be improved nonsignificantly following supplementation of PhytoGrow. The egg strength improved by 0.34% and 1.45% in the birds supplemented with PhytoGrow compared with those supplemented with Probiotic and Butyric acid, respectively. The Haugh unit increased by 1.15 and 0.03 in the group supplemented with PhytoGrow compared with those that received Probiotic and Butyric acid, respectively. Similarly, eggshell weight improved by 0.06 and 0.10 g/egg in the birds that received PhytoGrow supplementation compared with that in Probiotic and Butyric acid groups, respectively. Furthermore, eggshell defect percentage decreased by 0.19% and 0.11% following supplementation of PhytoGrow compared with Probiotic and Butyric acid groups, respectively (Table 4).

Table 2: Effect of PhytoGrow on Cumulative Production Performance Parameters of WL Layers Birds

Group	HDPE (%)	FI (g)	FI/egg	FCR	BW (g)	EW (g)
Phyto Grow	93.27 ± 0.89	93.30 ± 0.93	100.00 ± 0.71	1.72 ± 0.01	1471 ± 23.41	58.21 ± 0.26
Probiotic	92.40 ± 1.21	93.32 ± 0.75	101.00 ± 0.73	1.74 ± 0.01	1446.00 ± 12.97	58.05 ± 0.24
Butyric acid	92.72 ± 0.94	94.48 ± 0.27	102.00 ± 0.99	1.75 ± 0.02	1466.00 ± 22.17	58.31 ± 0.24
Mean	0.396	0.298	0.349	0.006	8.612	0.109
N	9	9	9	9	9	9
p-value	0.637	0.070	0.158	0.232	0.585	0.684

Expression of values as mean ± SEM; $n=9$.
$P>0.05$ compared with G3 based on one-way ANOVA followed by Dunnett's multiple comparison post hoc test.
Abbreviations: BW: body weight; EW: egg weight; FCR: feed conversion ratio;
FI: feed intake; HDEP: hed day egg production

Table 3: Effect of PhytoGrow on Egg Quality Parameters

Group	Density (g/cm^3)	Strength {dynes/ cm² (N)}	Haugh Unit	Shell Weight (g/egg)	ESD (%)
Phyto Grow	1.06 ± 0.00	29.29 ± 1.70	63.36 ± 1.17	5.86 ± 0.07	0.38 ± 0.03
Probiotic	1.05 ± 0.00	28.95 ± 1.66	62.21 ± 1.34	5.80 ± 0.13	0.57 ± 0.09
Butyric acid	1.07 ± 0.02	27.84 ± 1.22	63.33 ± 1.03	5.76 ± 0.09	0.49 ± 0.06

Expression of values as mean ± SEM; $n=7–9$.
$P>0.05$ compared with G3 based on one-way ANOVA followed by Dunnett's multiple comparison post hoc test.
Abbreviation: ESD: eggshell defects.

The pathogenic microbial (*E Coli* and *C perfringens*) load analysis was done to assess the effect of supplementation of PhytoGrow on microbial load in the ileum and cecum. In the cecum, *E Coli* ($P < .05$) and *C perfringens* ($p<0.001$) count significantly reduced in the birds that received PhytoGrow supplementation compared with those that received Butyric acid. Whereas in the ileum, *E Coli* and *C perfringens* count decreased in the birds that received PhytoGrow supplementation compared with those that received Probiotic and Butyric acid (Table 4).

Table 4: Effect of replacing Probiotics and Butyric acid with PhytoGrow on *E Coli* and *C perfringens* Count in the Ileum and Cecum of WL Layers

Group	*E coli* (cfu/100mL)		*C perfringens* (cfu/100mL)	
	Ileum	Cecum	Ileum	Cecum
PhytoGrow	4.52 ± 0.38	4.30 ± 0.42	5.61 ± 0.36	5.04 ± 0.24
Probiotic	4.58 ± 0.36	5.12 ± 0.33	5.66 ± 0.32	5.44 ± 0.33
Butyric acid	4.89 ± 0.42	5.71 ± 0.25[a]	6.45 ± 0.24	6.66 ± 0.22[b]

Expression of values as mean ± SEM; *n*=9.
[a]$p<0.05$ and [b]$p<0.001$ compared with G3 based on one-way ANOVA followed by Dunnett's multiple comparison post hoc test.

IV. DISCUSSION

All the egg production and egg quality variables were not affected by replacing probiotics and Butyric acids with herbal feed supplement included in the study. This indicates the potential possibility of using PhytoGrow / herbal compounds included in the product PG could able to maintain the layer performance similar to those fed Probiotics and Butyric acids. Herbs such as *A indica*, *C longa*, *A paniculata*, and *C zeylanicum* individually have conducive effects on production performance, egg quality traits, nutrient retention, and gut microbiota in commercial laying birds. However, the synergistic effects of a mixed formulation on layer birds were not studied. Hence, in the present study, the effect of PhytoGrow, which comprises *A indica*, *C longa*, *A paniculata*, and *C zeylanicum,* on production performance parameters, egg quality traits, and gut microbiota in WL layer birds were evaluated.

Our study results depicted that there was a nonsignificant improvement in the performance parameters such as HDEP, FI, FI/egg, FCR, BW, and EW in WL layer birds following PhytoGrow supplementation compared with Probiotics and Butyric acids. The pathogenic microbial (*E Coli* and *C perfringens*) load analysis revealed that *E Coli* ($p<0.05$) and *C perfringens* ($p<0.001$) count significantly reduced in both the cecum and ileum after PhytoGrow supplementation, compared with the birds that received Probiotics and Butyric acids. Furthermore, the egg quality parameters such as egg density, egg strength, Haugh unit, eggshell weight, and eggshell defects in the birds improved nonsignificantly following PhytoGrow supplementation. The conducive effects of PhytoGrow on growth performance, egg quality traits, and gut microbiota could be attributed to the synergistic effects of the phytoactive present in the herbal ingredients.

There is a resurgence to develop new strategies to boost poultry health. Balancing of intestinal microbiota (both harmful and beneficial bacteria) is essential for a healthy gut [14]. Many studies have proved the potential antimicrobial property of cinnamon essential oil [15], [16]. A study conducted by Yang *et al*. [16] indicated that broiler diets supplemented with cinnamon essential oil (100 mg/kg feed) significantly decreased cecal *E Coli* relative multiplicity and significantly increased cecal *Bifidobacterium* and *Lactobacillus* relative multiplicity. Hence, cinnamon essential oil could be a potential alternative to antibiotics (aureomycin). Another study conducted by Mehdipour and Afsharmanesh [15] on quails showed that the ileal coliform count decreased, and the ileal *Lactobacillus* count increased in quails that were fed cinnamon oil (200 ppm/kg diet) compared with control groups that were fed an antibiotic (virginiamycin) and cinnamon powder. A research study by Gupta *et al*. [17] found that the inhibitory effect of cinnamon essential oil against bacteria and fungi was very significant, and the lowest MIC was 1.25% (v/v) against bacteria's *Bacillus* sp *Klebsiella* sp., *E Coli*, and *Listeria monocytogenes* and fungi *Rhizomucor* sp. In addition, Abd El-Hack *et al*. [18] reported that the use of cinnamon essential oil as a feed additive in poultry diets had some beneficial effects on the growth performance and immunity parameters.

Wang *et al*. [19] indicated that the addition of 100 and 200 mg/kg turmeric extract to broiler's diet improved daily FI, whereas daily FI decreased with 300 mg/kg of the turmeric extract. The results of growth performance in our study were comparable to several other types of the research reported in the literature [20], [21]. Furthermore, Durrani *et al.* [22] reported an increase in BW with the addition of turmeric as it can stimulate protein synthesis. Their study results also mentioned that FI tended to be higher in the control group, and FCR was the lowest in the group that was treated with 0.5% turmeric. According to Wang *et al.* [19], and increased secretion of enzymes such as amylase, trypsin, chymotrypsin, and lipase might have improved FCR in birds supplemented with turmeric. Moreover, Chatterjee *et al.* [23] reported that the increase in FI might be because of the special aroma of turmeric, which is, in turn, because of volatile essential oil that comprises 1:8 cineole, zingiberene, β-sesquiphellandrene, α-phellandrene, turmerone, ar-turmerone, ar-curcumene, dehydrozingerone, and curlone.

In our study, there was an improvement in egg production ($p>0.05$) and EW, which is in accordance with other studies [24]-[26]. However, our study does not comply with studies conducted by Malekizadeh *et al.* [21] and Park *et al.* [27]. According to Malekizadeh *et al.* [21] study, egg production increased significantly ($p<0.05$), whereas EW decreased nonsignificantly in layer birds that were administered a diet with turmeric powder. They opine that turmeric might enhance digestive tract performance in layer birds, which results in improved egg production [21]. Park *et al*. [27] demonstrated that supplementation of turmeric powder increased egg production significantly, but not EW. Another study by Radwan *et al*. [28] found that egg production and egg mass increased, followed by the addition of turmeric, which might be because of improvement in uterus media (especially the site of calcium deposit) and thereby resulted in enhanced shell weight and thickness. Park *et al.* [27] also reported that the Haugh unit of the group fed with 0.25% turmeric was the highest among all groups ($p<0.05$). However, Radwan *et al.* [28] study did not report improvement in the Haugh unit despite adding turmeric to hen ration.

Supplementation of *A indica* with commercial poultry diet showed a favorable response in terms of nutrient conversion efficiency in a study by Chakravarty *et al.* [29], a similar kind of results were observed by Ma *et al*. [30] Chatterjee and Agrawala [31] reported that supplementation of *A indica* and *A paniculata* improved gastrointestinal micro-environment of the birds, which further resulted in increased utilization of nutrients and in turn increased productivity. A research study by Abdulla *et al.* [32] further supports these findings. Additionally, Esonu *et al.* [33] reported that the egg production improvement in layer birds due to the antioxidant property of *A indica*. Although Maliwan and Suksupath [34] reported that supplementation of *A paniculata* powder in layer bird's diets does not cause any significant effect on ($p>0.05$) on egg production performance, egg quality, and survival rate, supplementation of a higher dose of *A paniculata* powder increased yolk weight percent and yolk color [34].

V. CONCLUSION

In conclusion, this study demonstrated that dietary supplementation of PhytoGrow a blend herbal extract (*A indica*, *C longa*, *A paniculata*, and *C zeylanicum*) significantly improved gut health by reducing the pathogen count (*E Coli* and *C perfringens*) and numerically improved the egg production and egg quality variables compared to those fed probiotic or butyric acid in WL layer (42 to 53 weeks of age) diet.

Acknowledgments

The authors acknowledge Dr. Rajesh Kumawat, Head - Medical Services & Clinical Development, for his guidance and encouragements, Dr. Jayashree B Keshav and team, Scientific Publications Division, The Himalaya Drug Company, Makali, Bengaluru, for the editorial support.

Conflicts of interest

None to declare.

VI. REFERENCES

[1] "*The European Parliament and the Council of the European Union*". Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. Off J Eur Union, Vol. L268, pp. 29–43, 2003.

[2] H C Neu. "*The crisis in antibiotic resistance*". Sci, Vol. 257(5073), pp. 1064–1073, 1992.

[3] M Bozkurt, F "*Hippenstiel and A A Abdel-Warmth, SKehraus, K Küçükyilmaz, et al. Effects of selected herbs and essential oils on performance, egg quality and some metabolic activities in laying hens–A review*". Eur Poult Sci, Vol. 78, pp. 1-5, 2014.

[4] V H Varel. "*Livestock manure odor abatement with plant-derived oils and nitrogen conservation with urease inhibitors: A review*". J Anim Sci, Vol. 80(E-suppl_2), pp. E1–E7, 2002.

[5] A Brenes and E Roura. "*Essential oils in poultry nutrition: Main effects and modes of action*". Anim Feed Sci Tech, Vol. 158(1–2), pp. 1–14, 2010.

[6] M M Cowan. "*Plant products as antimicrobial agents*". Clin Microbiol Rev, Vol. 12(4), pp. 564–582, 1999.

[7] W J Craig. "*Health-promoting properties of common herbs".* Am J Clin Nutr, Vol. 70(3 Suppl), pp. 491S–499S, 1999.

[8] W Windisch, E Rohrer, and K Schedule. "*Phytogenic feed additives to young piglets and poultry: mechanisms and applications*". In: Steiner T, ed. Phytogenics in Animal Nutrition: Natural Concepts To Optimize Gut Health and Performance. Nottingham, UK: Nottingham University Press; pp. 19–39, 2009.

[9] H Greathead. "*Plants and plant extracts for improving animal productivity".* Proc Nutr Soc, Vol. 62(2), pp. 279–290, 2003.

[10] S A Burt and R D Reinders. "*Antibacterial activity of selected plant essential oils against*" Escherichia coli O157: H7. Lett Appl Microbiol, Vol. 36(3), pp. 162–167, 2003.

[11] F Hippenstiel, AA Abdel-Wareth, S Kehraus and K H Südekum. "*Effects of selected herbs and essential oils, and their active components on feed intake and performance of broilers–a review".* Arch Geflügelk, Vol. 75(4), pp. 226–234, 2011.

[12] M Bozkurt, F Hippenstiel, A A Abdel-Wareth, S Kehraus, K Küçükyilmaz, and K H Südekum. "*Effects of selected herbs and essential oils on performance, egg quality, and some metabolic activities in laying hens – A review*". Eur Poult Sci, Vol. 78, pp. 1–5, 2014.

[13] C H Collins and PM Lyne. *Microbiological Methods*. 3rd ed. Arnold, a member of the Hodder Headline Group, London, 1970.

[14] S S Elnesr, M Alagawany, H A Elwan, M A Fathi, M R Farag. "*Effect of sodium butyrate on intestinal health of poultry–a review*". Ann Anim Sci, Vol. 20, pp. 29–41, 2020.

[15] Z Mehdipour and M Afsharmanesh. "*Evaluation of synbiotic and cinnamon (Cinnamomum Verum) as antibiotic growth promoter substitutions on growth performance, intestinal microbial populations, and blood parameters in Japanese quail*". J Livestock Sci Technol, Vol. 6(2), pp. 1–8, 2018.

[16] Y F Yang, L L Zhao, Y X Shao, X D Liao, L Y Zhang, L U Lin and X G Luo. "*Effects of dietary graded levels of cinnamon essential oil and its combination with bamboo leaf flavonoid on immune function, antioxidative ability and intestinal microbiota of broilers*". J Integr Agric, Vol. 18(9), pp. 2123–2132, 2019.

[17] C Gupta, A P Garg, R C Uniyal, and A Kumari. "*Comparative analysis of the antimicrobial activity of cinnamon oil and cinnamon extract on some food-borne microbes*". African J Microbiol Res, Vol. 2(9), pp. 247–251, 2008.

[18] M E Abd El-Hack, E Mohamed, M Alagawany, A M Abdel-Moneim, N G Mohammed, A F Khafaga, M Bin-Jumah, S I Othman, A A Allam and S S Elnesr. *"Cinnamon*

(Cinnamomum zeylanicum) oil as a potential alternative to antibiotics in poultry". Antibiotics, Vol. 9(5), p. 210, 2020.

[19] D Wang, H Huang, L Zhou, W Li, H Zhou, G Hou, J Liu, and L Hu. "*Effects of dietary supplementation with turmeric rhizome extract on growth performance, carcass characteristics, antioxidant capability, and meat quality of Wenchang broiler chickens*". Italian J Anim Sci, Vol. 14(3), p. 3870, 2015.

[20] S Ghazi, M Malekizadeh, and M M Moeini."*The effects of different levels of ginger and turmeric rhizomes powder on productive performance characteristics of laying hens*". Euphrates J of Agriculture Sci, Vol. 3(9), pp.1-5, 2011.

[21] M Malekizadeh, M M Moeini, and S Ghazi. "*The effects of different levels of ginger (Zingiber officinale Rosc) and turmeric (Curcuma longa Linn) rhizomes powder on some blood metabolites and production performance characteristics of laying hens*". J Agr Sc Technol, Vol. 14, pp. 127–134, 2012.

[22] F R Durrani, M Ismail, A Sultan, S M Suhail, N Chand, and Z Durrani. "*Effect of different levels of feed added turmeric (Curcuma longa) on the performance of broiler chicks*". J Agr Biol Sci, Vol. 1(2), pp. 9–11, 2006.

[23] S Chatterjee, P S Variyar, A S Gholap, SR Padwal-Desai, and D R Bongirwar. "*Effect of γ-irradiation on the volatile oil constituents of turmeric (Curcuma longa).*" Food Res Int, Vol. 33(2), pp. 103–106, 2000.

[24] T Incharoen and K Yamauchi. "*Production performance, egg quality, and intestinal histology in laying hens fed dietary dried fermented ginger*". Int J Poult Sci, Vol. 11, pp. 1078–1085, 2009.

[25] C Laganá, C C Pizzolante, E S Saldanha, and J E de Moraes. "*Turmeric root and annato seed in second-cycle layer diets: performance and egg quality*". Braz J Poult Sci, Vol. 13(3), pp. 171–176, 2011.

[26] X Zhao, Z B Yang, W R Yang, Y Wang, S Z Jiang, and G G Zhang. "*Effects of ginger root (Zingiber officinale) on laying performance and antioxidant status of laying hens and on dietary oxidation stability*". Poult Sci, Vol. 90(8), pp. 1720–1727, 2011.

[27] S S Park, J M Kim, E J Kim, H S Kim, B K An, and C W Kang. "*Effects of dietary turmeric powder on laying performance and egg qualities in laying hens.*" Korean J Poult Sci, Vol. 39(1), pp. 27–32, 2012.

[28] R Nadia, R A Hassan, E M Qota, and H M Fayek. *"Effect of natural antioxidants on oxidative stability of eggs and productive and reproductive performance of laying hens*". Int J Poult Sci, Vol. 7(2), pp. 134–150, 2008.

[29] A Chakravarty and J Prasad. "*Study on the effect of neem leaf extract and neem cake extract on the performance of broiler chicks*". Poult Adv, Vol. 24, pp. 37–38, 1991.

[30] D Ma, A Shan, Z Chen, J Du, K Song, J Li, and Q Xu. "*Effect of Ligustrum lucidum and Schisandra Chinensis on the egg production, antioxidant status, and immunity of laying hens during heat stress*." Arch Anim Nutr, Vol. 59(6), pp. 439–447, 2005.

[31] S Chatterjee and S K Agrawala. "*From the concept of the simple liver tonic of the previous generation to "Livoliv": a new generation poultry performance-enhancing system*". Phytomedicine, Vol. 3, pp. 13–25, 2002.

[32] M A Abdulla, K A, Ahmed, F H Al-Bayaty, and Y Masood. "*Gastroprotective effect of Phyllanthus niruri leaf extract against ethanol-induced gastric mucosal injury in rats*". Afr J Pharm Pharm, Vol. 4(5), pp. 226–230, 2010.

[33] B O Esonu, C Okoli, M Opara, O Obikaonu, C Udedibie, and M Iheshiulor. "*Physiological response of laying birds to Neem (Azadirachta indica) leaf meal-based diets: bodyweight organ characteristics and hematology*". Online J Health Allied Sci, Vol. 2, p. 4, 2006.

[34] K Maliwan and K Suksupath. "*Effects of Andrographispaniculata (Burm. f.) Wall. Ex nees supplementation in laying hen rations on production performances and egg quality*". Poster presented at Proceedings of 53rd Kasetsart University Annual Conference; February 3–6; Kasetsart University, Thailand, 2015.