

The Efficacy of *Allium sativum* and *Phyllanthus amarus* Extracts on The Performance of Hens

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Abstract

The efficacy of commercial products from Allium sativum (Biosol Immune A) and Phyllanthus amarus extracts (Biosol Diep Ha Chau) on productive performance and egg quality in laying hens were determined in this study. A total of 1584 Isa Brown hens were randomly assigned to 2 groups in a completely randomized one-factor design. In the control group, layers were supplemented the doxycycline via drinking water while the tested group was added the extract of the two mentioned herbs. The results showed that both groups had almost the same laying rate, average egg weight, feed conversion ratio, and mortality rate. For egg quality, the eggs of herbal-used layers having Haugh units and albumin ratio higher than that of antibiotic-used layers ($p < 0.05$). However, there was no statistically significant difference between herbal and control groups in shape criteria, yolk ratio, shell thickness, yolk colour, and shell colour ($p > 0.05$). This study suggested that Biosol Immune A and Biosol Diep Ha Chau can be used as antibiotic alternative for layers.

Keywords: Extract, Allium Sativum, Phyllanthus Amarus, Layer, Performance.

Introduction

Antibiotic used in animal production contribute to development of antibiotic resistance among bacteria. A publication in 2020 has reported that antibiotic use for animal in Vietnam was 247 mg/kg which was higher than that in European countries (151.1mg/kg). Government has implemented several regulations as national action plan to reduce antibiotic use such as Animal Husbandry Law (2018) to ban antibiotic as growth promotor in feed. Circular No. 12/2020/TT0BNNPTNT of the Ministry of Agriculture and Rural Development specifying the prescribing of veterinary drugs was issued on November 9, 2020 to restrict use of antibiotic classes in disease prevention. Therefore, scientists and industries must contribute to the process of education and providing alternative measures for farmers to maintain animal health as well as to prevent infections in new situation.

According to Mirzaei-Aghsaghali, 2012, herbal extracts can increase palatability, improve the release of endogenous digestive enzymes, activate the immune system, against bad bacteria and viruses and antioxidants. Allicin in garlic (*Allium sativum*) extract has bactericidal effect, stimulates digestion, sulfur compounds and polyphenols have high biological activity (Rahman et al., 2012). Garlic will enhance the performance of laying hens and improve egg quality if it is added to the feed (Lim et al., 2006). *Phyllanthus* extract has protective and supportive effects on liver function (Chattopadhyay et al., 2006; Pramyothin et al., 2007), immunostimulant and antibacterial effects (Taiwo et al., 2009). Our recent report (Pham et al., 2022) has proved that extract of *Allium sativum* from alcohol inhibited *Ornithobacterium rhinotracheale* very well and *Phyllanthus* extract has bactericidal effect against *E. coli* ATCC 25922 with MBC of 250 mg/ml. Diseases caused by *Ornithobacterium rhinotracheale* and *Escherichia coli* are one of the causes of significant financial losses, increased mortality, reduced productivity, and increased elimination rates (Umali et al., 2018 and Barnes et al., 2003). To prevent diseases caused by bacteria, layer producers in Vietnam often use doxycycline, a broad-spectrum antimicrobial. Therefore, this study evaluated the efficacy of commercial products from *Allium sativum* (Biosol Immune A, Vietnam) and *Phyllanthus amarus* extracts (Biosol Diep Ha Chau, Vietnam) on productive performance and egg quality in Isa Brown laying hens with target of using herbal products as antibiotic alternative.

Materials and Methods

The experiment was conducted on 1584 Isa Brown laying hens with the age of 23 – 29 weeks, originated from CP Viet Nam Company to a commercial farm in Dong Nai province in Vietnam. This farm operated with close system and climate was automated control to get the optimum condition for layers. The feed used for layers in both groups was Hi-Gro 524 bran of CP Vietnam (Table 1). Feeding time was the same at 7 am and 2 pm every day. Layer was fed using the linear chain feeder. This farm applied the control diet plan with the average feed used by layers during the 23–29-week period was 110g/head/day. The drinking water system of the farm consisted of a stock water tank and a secondary water tank. In the layer house, each row had a small water tank and a convenient valve system for the experiment of supplementation the liquid herbal medicine to the drinking water. The layer consumed water via the bell drinkers.

Table 1. Hi-Gro 524 bran feed for layer calculated nutrient composition

Nutrition	Unit	Value
Humidity (max)	%	14
Crude protein (min)	%	17
Fiber (max)	%	6
ME (tối thiểu)	Kcal/Kg	2700
Calcium (min - max)	%	3,4 - 4,8
Total phosphorus (min - max)	%	0,4 - 1
Lysine (min)	%	0.7
Methionine + Cystine (min)	%	0.6
Antibiotic: none		

The birds were randomly assigned into two treatments of a completely randomized one-factor fashion. Each group contained 198 cages; each cage had 4 laying hens. Layers were introduced into the experiment at 17 weeks of age and lasted until 29-week-old. The herbal group used *Phyllanthus* extract (Biosol Diep Ha Chau, Vietnam) at a dose

of 2.0 ml/L in drinking water for 2 days, every 2 weeks and garlic extract (Biosol Immune A, Vietnam) at a dose of 1.0 ml/L drinking water for 7 consecutive days, every two weeks. The volume of drinking water of layers in the house during period of 2 hours was measure, then the supplementation of the herbal products will be added. The both herbal products in this study was in liquid form in which Biosol Diep Ha Chau was *Phyllanthus* aqueous extract and Biosol Immune A was alcohol-based garlic extract. The control group was supplemented with doxycycline 50%, for 3 consecutive days once a month at a dose of 1 kg/20 tons body weight (50mg/kg) via drinking water as the routine procedure of the farm.

Egg quantities were recorded daily and the egg-laying rate was calculated. Evaluate time lasted from 23 to 30 weeks old of the layers. Fifty eggs of each group were collected to measure the criteria for egg quality using the yolk colour meter, eggshell colour range, digital caliper, digital thickness gage and digital electronic scale. Egg quality were measured within the first 24 hours after collected from the farm at the Department of Animal Production, Faculty of Animal Science and Veterinary Medicine, Nong Lam University, Ho Chi Minh City.

To examine the antibiotic residue, eggs were collected from the doxycycline group for four time point (1) eggs from layers that were using antibiotic; (2) eggs from layers that stopped taking antibiotic 4 days; (3) eggs from layers that stopped using antibiotic 11 days; and (4) eggs from layers that stopped taking antibiotic 18 days. The samples were tested using CH104 – LC-MS/MS (UpScience, Vietnam) following the protocol of AOAC 995.09.

Results

Percentage Hen Day Production

Table 2. Percentage hen day production via weeks (%)

Age in week	Group		SEM	P
	Control	Herbal		
20	64.09	61.71	1.27	0.37
21	75.20	73.05	2.28	0.66
22	84.19	85.95	0.83	0.31
23	86.23	87.21	0.69	0.49
24	91.18	91.36	0.93	0.93
25	94.92	95.89	0.33	0.15
26	95.38	96.27	0.55	0.44
27	94.71	95.16	0.23	0.36
28	94.71	94.79	0.32	0.9
29	92.95	93.05	0.17	0.78
30	92.71	92.50	0.29	0.73
20-30	87.84	87.1	0.85	0.73

Data from Table 2 showed that the laying rate of 22-week-old Isa Brown hens in the control and herbal group was over 85% before reaching to the peak of over 92% at 30 weeks of age. Our results were slightly lower than the ISA Brown standard (95.9%) provided by Hendrix Genetics (2019). Percentage hen day production in the two groups was similar ($p>0.05$). Thus, supplementing with the commercial products of garlic and *Phyllanthus* extracts can replace doxycycline.

Mortality and Culling Rate

The percentage of mortality and culling rate in the control group was 0.63% higher than that of the herbal chickens that used herbs (0.51%). However, there was no significantly statistical difference ($p=0.738$).

Average Egg Weight

Table 3. Average egg weight via weeks (g/egg)

Weeks of age	Group		SEM	P
	Control (g/egg)	Herbal (g/egg)		
20	54.13	54.26	0.26	0.82
21	56.43	56.31	0.14	0.68
22	57.55	56.99	0.16	0.08
23	57.6	58.46	0.38	0.28
24	59.07	58.93	0.12	0.58
25	60.04	59.78	0.15	0.43
26	60.81	60.52	0.16	0.37
27	61.06	61.26	0.05	0.02
28	61.05	61.14	0.05	0.41
29	61.22	61.28	0.08	0.75
30	61.31	61.64	0.12	0.17
20-30	59.14	59.12	0.19	0.94

Results from Table 3 indicated that the average egg weight of the control group and that of the herbal group were equal although the figures in week 27 showed that herbal group's egg a bit better than the control group's eggs ($p < 0.05$). At 30 weeks of age, the average egg weight in the herbal and control groups was 61.64 g/egg and 61.31 g/egg, respectively. This result was similar to the announcement of Hendrix Genetics (2020) for ISA Brown which was 61.6 g/egg.

This study has similarities with some researchers from Novi Sad University (Serbia) who confirmed that garlic had a good effect of promoting weight gain of chickens (Issa et al., 2012). Garlic supplemented in feed that enhanced the performance of laying hens and improved egg quality (Lim et al., 2006; Yalcin et al., 2006; Khan et al., 2007).

Egg Quality Parameters

Table 4. Egg quality parameters

Weeks of age	Group		SEM	P
	Control Group	Test Group		
		Shape Index		
23	0.80 ^a	0.80 ^a	0.003	0.07
25	0.80 ^a	0.79 ^a	0.004	0.33
27	0.80 ^a	0.81 ^a	0.003	0.74
29	0.79 ^a	0.79 ^a	0.003	0.76
23 – 29	0.80 ^a	0.79 ^a	0.003	0.17
		Haugh unit		
23	91.93 ^a	92.71 ^a	0.72	0.44
25	90.50 ^a	90.42 ^a	0.63	0.93
27	89.53^b	91.37^a	0.59	0.03
29	89.52^b	91.45^a	0.63	0.03
23 – 29	90.37^b	91.49^a	0.65	0.01
		Yolk (%)		
23	23.27 ^a	22.82 ^a	0.19	0.09
25	23.56 ^a	23.56 ^a	0.24	0.96
27	24.70 ^a	24.75 ^a	0.30	0.90
29	25.49 ^a	25.77 ^a	0.28	0.48
23 – 29	24.26 ^a	24.23 ^a	0.29	0.87
		Albumen (%)		

23	37.38^b	41.48^a	0.87	0.001
25	38.80 ^a	39.82 ^a	0.90	0.42
27	39.45 ^a	39.25 ^a	0.85	0.87
29	40.24 ^a	41.08 ^a	0.35	0.55
23 – 29	38.97^b	40.41^a	0.91	0.03
Eggshell (%)				
23	12.50 ^a	12.43 ^a	0.10	0.61
25	12.53^a	12.21^b	0.10	0.03
27	12.13 ^a	12.32 ^a	0.10	0.14
29	12.14 ^a	12.22 ^a	0.10	0.60
23 – 29	12.33 ^a	12.30 ^a	0.10	0.68
Eggshell thickness (mm)				
23	0.37 ^a	0.37 ^a	0.003	0.85
25	0.37 ^a	0.37 ^a	0.003	0.38
27	0.38^a	0.36^b	0.003	0.000
29	0.39^b	0.40^a	0.003	0.03
23 – 29	0.38 ^a	0.37 ^a	0.003	0.55
Yolk color				
23	12.50 ^a	12.24 ^a	0.16	0.24
25	10.04^b	10.80^a	0.20	0.01
27	9.42 ^a	9.08 ^a	0.18	0.19
29	9.38 ^a	9.74 ^a	0.16	0.11
23 – 29	10.34 ^a	10.47 ^a	0.25	0.45
Shell color				
23	6.90 ^a	7.14 ^a	0.11	0.31
25	6.60 ^a	6.62 ^a	0.13	0.92
27	6.22^b	6.78^a	0.13	0.003
29	6.70 ^a	6.34 ^a	0.13	0.05
23 – 29	6.63 ^a	6.72 ^a	0.13	0.31

*Different letters in the same row indicate significant difference with $p < 0.05$

Results in the Table 4 indicated interestingly that herbal group had a higher Haugh unit than the control group over the entire period ($p=0.01$). According to the research results of the company Hendrix genetics, the Haught unit was 82. According to the national standard TCVN 1858:2018, eggs of type AA were eggs that had to have a Haugh unit ≥ 72 and class A eggs had a Haugh unit in range of 60 to < 72 . Therefore, eggs in this experiment were in good quality. The herbal supplementation also resulted in higher percentage of egg white in the test group (40.41 %) than that in the control group (38.97 %) with $p=0.03$ for the whole period.

There was no difference between the shape index, the percentage of yolks, the percentage of eggshell, eggshell thickness, yolk colour and shell colours in control group and herbal group in the total period of experimental time although the herbal group showed some advantage over the control group in some weeks (Table 4).

According to previous study for Isa Brown egg quality (Anene et al, 2020), egg shape index should be in the range of 71-79%; Haugh unit should be around 83 to 100; yolk percentage should be from 18-23%; the percentage of eggshell accounted should be 9-13% of egg weight; the eggshell thickness ranged from 0.3-0.4 (mm); yolk color was 11. Therefore, the eggs from both group in this experiment met the good quality of Isa Brown eggs. Hence, garlic and *Phyllanthus* extracts helped hens produce the good-quality eggs.

Feed Conversion Ratio

From 21 to 30 weeks of age, when the laying rate was over 80%, the feed conversion ratio of the 2 experimental groups ranged from 1.93 to 2.16 (Table 5). This result was consistent with the study of Hendrix Genetics company (2.14 kg feed/kg egg) on feed conversion ratio.

Table 5. Average weekly feed conversion ratio (kg feed/kg egg)

Weeks of age	Group		SEM	P
	Control group (Kg feed/Kg egg)	Herbal group (Kg feed/Kg egg)		
20	2.76	2.85	0.07	0.51
21	2.39	2.47	0.08	0.64
22	2.15	2.13	0.02	0.62
23	2.16	2.11	0.02	0.28
24	1.99	1.99	0.01	0.82
25	1.98	1.97	0.02	0.82
26	1.93	1.93	0.01	0.62
27	1.95	1.93	0.01	0.24
28	1.99	1.99	0.01	0.99
29	2.04	2.04	0.004	0.92
30	2.04	2.04	0.01	0.94
20-30	2.13	2.13	0.02	0.87

The feed conversion ratio of control group and herbal group was 2.13 kg /kg and 2.14 kg/kg, respectively. This difference among groups was not statistically significant ($p>0.05$). This showed that using herbs in the experiment helped chickens digest food well, ensure health, and did not affect the laying rate and egg quality.

Antibiotic Residues in Eggs

Table 6. Doxycycline residue in eggs during time points

	Timepoint (After N Day withdrawal)			
	1 (0D)	2 (A4D)	3 (A11D)	4 (A18D)
µg/kg	1910	384.5	non	Non

Data shown in Table 6 indicated that doxycycline residue in the eggs collected from layers that being treated with doxycycline was at 1910 µg/ml. After 4 days of stopping using the antibiotic, the doxycycline residue was 384.5 µg/ml. In Viet Nam, according to Circular No. 24/2013/TT-BYT, the antibiotics chlortetracycline, oxytetracycline and tetracycline had residue levels in egg-laying hens regulated below 400 µg/kg. The doxycycline residue in eggs at 4 days withholding period in our study was much higher than the rule.

The MRL of doxycycline in egg was 200 µg/kg as of the EU regulation. Previous study showed that antibiotics could survive in yolk for 27 days and white during 25 days since adding doxycycline to the drinking water of laying hens within 7 days Yoshimura et al. (1991). Therefore, China and the EU have banned the use of doxycycline during the egg-laying period (Guo et al., 2021).

Conclusion

This is the first study to prove that supplementation of product from extract of *Allium sativum* and *Phyllanthus amarus* in the drinking water of laying hens from 17 to 29 weeks of age that increased the Haugh index, and albumin index higher than that of the control group. Other parameters such as of daily feed consumption, laying rate, average egg weight, feed conversion ratio, mortality, elimination, shape index, yolk ratio, shell ratio, shell thickness, yolk color and shell color of the eggs in the herbal group were similar to the doxycycline group. Doxycycline was detected in the eggs of layers in the group using doxycycline for up to 4 days after the last day using the antibiotic. *Allium sativum* extract (Biosol Immune A) and *Phyllanthus amarus* extract (Biosol Diep Ha Chau) can be antibiotic alternatives for Isa Brown layers.

Contribution by Authors

Equal contribution

Conflict of Interests

There is no conflict of interest.

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