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The impact of avian IgY antibodies and probiotics supplementation via drinking water on growth and laying performance of 16-week-old layer pullets

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ABSTRACT

Antibiotics raise an issue concerning the development of antibiotic resistance of some pathogenic bacteria. The use of probiotics and Avian IgY in layer pullet farming is a better way of protecting against pathogenic bacteria and increasing production performance. Supplementation of Avian IgY antibodies and probiotics (Lactobacillus acidophilus, Streptococcus faecium, Bifido bacterium longghum, Bacillus thermophilum) were conducted on 5,724 healthy layer pullets to determine its effect on their growth and laying performance. The supplement was in powder form with a dosage of 100 mg per liter of water for 6 hours. The effect of the supplementation was evaluated by measuring production parameters on growth and laying performance of the layer pullets; there were 3 treatment groups, the control group, Treatment 2 which received 5 days initial supplementation for the first week and then twice every week for 9 weeks and Treatment 3 which received 5 days initial supplementation for the first week and then twice every 2 weeks for 9 weeks. Results showed that the supplementation had significant effects in the age in days when 50% of the total population per treatment laid their first eggs, average number of eggs produced, % Hen day production, % Hen house production, total weight of eggs, and feed conversion efficiency. It also showed that the supplementation is economical and yielded higher profit. On the other hand, the supplementation had no significant effect on the average body weight and mortality count of the layer pullets. The supplementation of Avian IgY antibodies and probiotics in water soluble powder via drinking water with 5 days initial supplementation of Avian IgY antibodies and probiotics for the first week; then twice every 2 weeks for 9 weeks has the greatest effects to increase the production performance and profitability of layers.

Introduction

The Poultry Industry is one of the Philippine economy's biggest and most progressive components. It is largely made up of two divisions: the broiler industry and the layer industry. Although, Poultry also includes smaller players like the quail, turkeys, and other fowl-based ventures. Eggs produced by the Poultry industry play a vital role in providing staple food supply (Papanikolaou and Fulgoni, 2019) not only here in the Philippines but for the whole world. An important significance of this study is to help poultry farmers increase their layer production thus also helping the upliftment of poultry industry.

To this day, it is a given fact that the use of antibiotics in the poultry layer industry is customary on almost all farms (Mohammadzadeh *et al*., 2022). Some antibiotics commonly used in philippines such as tetracycline, amoxicillin, erythromycin, gentamicin (Barroga *et al*., 2020) together with other feed additives are also used to increase the production performance of layer pullets (The Philippines Recommends for Table Egg Production, 2008). However, the use of antibiotics raises an issue concerning the development of antibiotic resistance of some pathogenic bacteria such as *Escherichia coli* (Sebastian *et al*., 2021); *Listeria monocytogenes* (Panera-Martinez *et al*., 2022); and Typhoidal *Salmonella* (Yin

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et al., 2021). Due to this occurrence, farm owners have an emerging trend in using a more natural way of giving their pullets protection against pathogenic bacteria.

The use of probiotics in layer pullet farming is becoming to be known as a better way of protecting layer pullets against pathogenic bacteria and being able to help in the increase and development of the layer pullets' production performance (Ray *et al*., 2022; Kimminau *et al*, 2021). Immunoglobulins IgY constitutes an important fraction of egg yolk protein (equivalent to IgG in mammalian), which offers protection against pathogens to the embryo and chicks after birth (Gandarilla *et al*., 2022). IgY comprises 75% of all immunoglobulins in avian hosts, with concentrations of up to 5 mg/ml in serum, synthesized by plasma cells derived from B lymphocytes (Thirumalai *et al*., 2019). Importantly, IgY as an excellent source of polyclonal antibodies shows better immune responsiveness to mammalian antigens due to phylogenetic distance, and activates no adverse inflammatory responses, making IgY-based therapeutics more efficient and persistent against infections (Ahmadi *et al*., 2021).

The supplementation of Avian IgY antibodies together with probiotics in the drinking water of layer pullets will not only be able to help improve the layer pullets' performance but also to provide an alternative and a more "natural" way for poultry farmers in giving layer pullets adequate support in terms of its growth and laying performance. This study aims to investigate if the supplementation of Avian IgY antibodies and probiotics via the drinking water of 16-week-old Dekalb Single Comb White Leghorn pullets will influence the growth and laying performance of these layer pullets.

Materials and Methods

Animals

Five thousand seven hundred twenty-four (5,724) 16-week-old Dekalb Single Comb White Leghorn ready-to-lay pullets were used in the study. The pullets were raised in a conventional pullet grow-out farm in Malvar, Batangas, and then were transferred to the poultry layer house. Upon transfer, the cages were labeled with their corresponding treatment groups and the pullets were randomly distributed into the three treatment groups.

Avian IgY antibodies and probiotics

A water-soluble powder from Agranco Corporation, USA containing Avian IgY antibodies and probiotics (*Lactobacillus acidophilus*, *Streptococcus faecium*, *Bifido bacterium longghum*, *Bacillus thermophilum*) at a concentration of 90,000,000,000 CFU/kg and dosage of 50g/10,000 birds or 100 mg per liter of water for 6 hours was incorporated for the water supply of the layer pullets from Treatments 2 and 3.

Table 1. Experimental design for treatments.

Treatments	Diets
Treatment 1 (T1)	Basal water
Treatment 2 (T2)	basal water with 5 days initial supplementation of Avian IgY antibodies and probiotics for the first week; then twice every week for 9 weeks
Treatment 3 (T3)	basal water with 5 days initial supplementation of Avian IgY antibodies and probiotics for the first week; then twice every 2 weeks for 9 weeks

Table 2. Nutrient composition of grower layer basal diets used in the experiment.

Item	Contents
Ingredients (%)	
Maize	45.75
Soybean meal	36.19
Wheat	10.00
Soybean oil	3.82
Dicalcium phosphate	1.39
Limestone	1.32
Vitamin-mineral Premix*	0.50
DL methionine	0.53
L-lysine HCl	0.12
Salt	0.38
Nutrient composition	
Metabolizable Energy (kcal.kg)	3100
Crude Protein (%)	17.00
Lysine	0.52
Calcium	1.8
Phosporus, available	0.35

*vitamin-mineral premix contained the following per kg of diet: 9750 IU vitamin A; 2000 IU vitamin D3; 25 IU vitamin E; 2.97 mg vitamin K; 7.6 mg riboflavin;13.5 mg Dl Ca-pantothenate; 0.012 mg vitamin B12; 29.7 mg niacin; 1.0 mg folic acid, 801 mg choline; 0.3 mg biotin; 4.9 mg pyridoxine;2.9 mg thiamine; 70.2 mg manganese; 80.0 mg zinc; 25 mg copper; 0.15 mg selenium; 50 mg ethoxyquin; 1543 mg wheat middling's; 500 mg ground limestone.

Experimental design

The pullets were randomly distributed into three groups following a completely randomized design. There were 4 pullets per cage which are considered as the replicate, there were 477 replicates, and there was a total of 1,908 pullets per treatment. The treatment groups were distributed into 3 rows of cages. The Avian IgY antibodies and probiotics supplements were given through drinking water, with the aid of 3 separate water tanks with 1000L capacity each. The three treatment groups are shown in Table 1.

The experimentation period lasted for 10 weeks. All the layers in the experiment were subjected to the same management procedures and fed by a commercial feed (Table 2) which follows the nutrient requirements for layers that were set by PHILSAN. The pullets were fed laying mash for the duration of the feeding trial. The procedures mentioned were approved by the Institutional Animal Care and Use Committee.

Growth and laying performance

Average body weight was obtained per treatment group every week by randomly weighing 20 pullets per treatment group and obtaining their average weight. Mortality was noted by recording the number of dead pullets per cage, per treatment group.

Age in days when 50% of the total population per treatment laid their first eggs was noted by recording the eggs produced per replicate, per day, and getting its total. Egg production was noted by recording the number of eggs produced daily per cage, then getting the sum per week, and then computing the average egg production weekly per cage, per treatment group. % Hen day production was computed by dividing the number of eggs produced per Treatment by the number of hens per day and then multiplying it by 100. The percentage (%) of hen house production was computed by dividing the number of eggs produced per day by the number of pullets at the start of the experiment, then multiplying it by 100. The total weight of eggs produced per treatment was recorded with the aid of an egg grader (TD-101A egg grader machine) to weigh the eggs daily and every corresponding size is equivalent to a specific amount of weight in grams, pullets = 47g, peewee = 52g, small = 57g, medium = 62g, large = 67g, extra-large = 72g, jumbo = 77g. Then the total weight of eggs produced for the whole duration of the experiment was noted.

Feed conversion efficiency was computed by getting the total weight of feeds consumed per treatment and dividing it by the total weight of the eggs produced per treatment. The Income Over Feed, Chick, and Medication costs were computed by taking note of the total medication cost for 10 weeks and dividing it by the total number of pullets at the end of the experiment.

Statistical analysis

The data were analyzed using the procedures for general linear models of statistical analysis (SAS) involving Completely Randomized Design (CRD). The comparisons on mean daily egg production, body condition, feed conversion efficiency, egg weight, and mortality count between treatment groups were analyzed using Repeated Measures Analysis of Variance (MANOVA), Scheffe's Test, and Kruskal-Wallis Test at $P<0.05$

Results

The supplementation of the Avian IgY antibodies and probiotics had no significant effect on the average body weight of the pullets. Statistically, the result was shown in Table 3, there were no significant differences in the average body weight of the pullets among the treatment groups for the whole duration of the study of 10 weeks ($\alpha=0.05$). There were no notable differences (Table 4) in the mortality count of the treatment groups throughout the whole duration of the experiment except on week 4, wherein there was a sudden increase in the average mortality count per treatment group.

Table 3. The average body weight (gram) per treatment group of the Dekalb Single Comb White Leghorn layer pullets supplemented with Avian IgY antibodies and probiotics for 10 weeks.

Week	T1	T2	T3	Sig.
1	1059	1080	1085	0.4853^{ns}
2	1145.50	1129.50	1135.50	0.4413^{ns}
3	1258.75	1243	1254.50	0.6531^{ns}
4	1389	1415.50	1389.50	0.7492^{ns}
5	1455	1423.50	1428	0.3253^{ns}
6	1496	1463.50	1467	0.7492^{ns}
7	1561	1567.50	1564	0.9603^{ns}
8	160	1570.50	1548	0.1246^{ns}
9	1587.50	1597	1568	0.3773^{ns}
10	1581.50	1578	1574	0.8790^{ns}

ns = not significant *Significant at $\alpha<0.05$, **Significant at $\alpha<0.01$.

Table 4. The weekly mortality (chickens) count per cage, a treatment group of the Dekalb Single Comb White Leghorn layer pullets supplemented with Avian IgY antibodies and probiotics for 10 weeks.

Week	T1	T2	T3
1	0	0	0
2	1	1	1
3	4	4	2
4	8	10	7
5	1	3	4
6	1	1	2
7	1	1	0
8	1	1	0
9	1	0	0
10	1	0	0
Total	14	21	16

The Avian IgY antibodies and probiotics supplementation had a significant effect on the differences in age on days when 50% of the total population per treatment laid their first eggs. As seen in Table 5, showed that T3 had an earlier onset of laying for 50% of its population at 161 days, in comparison with the T2 at 166 days and Treatment 1 at 167 days. According to the production guide of ISA, the ideal age of onset of laying off 50% off the population for Dekalb Single Comb White Leghorn layer pullets is 161 days.

The Avian IgY antibodies and probiotics supplementation had a significant effect ($\alpha<0.01$) on the differences in egg production as seen in Table 6. The data presented was tabulated weekly; it was noted that the differences among the three treatment groups were highly significant from weeks 4 to 10 ($\alpha=0.01$). It was also noted that Treatment 3 had a significantly higher average weekly egg production throughout weeks 4 to 10 over the other two treatment groups ($\alpha=0.05$). In addition to that, Treatment 2 had a significantly higher average weekly egg production than treatment 1 in weeks 6 and 7 ($\alpha=0.05$).

Table 5. The age in days of the onset of laying off 50% per treatment group of the Dekalb Single Comb White Leghorn layer pullets supplemented with Avian IgY antibodies and probiotics.

Treatment	Age in days
Treatment 1	167
Treatment 2	166
Treatment 3	161

Table 6. The average weekly egg production (eggs) per cage, per treatment group of the Dekalb Single Comb White Leghorn layer pullets supplemented with Avian IgY antibodies and probiotics for 10 weeks.

Week	T1	T2	T3	Sig.
1	0	0	0	-
2	0	0	0	-
3	0.05	0.03	0.08	0.1046^{ns}
4	0.40	0.44	0.68*	0.0027**
5	1.96	2.24	2.99*	<0.0001**
6	5.08*	6.15*	7.53*	<0.0001**
7	8.60*	9.75*	11.79*	<0.0001**
8	12.90	13.55	16.32*	<0.0001**
9	17.01	17.72	19.97*	<0.0001**
10	20.26	20.67	22.30*	<0.0001**

ns = not significant *Significant at $\alpha<0.05$, **Significant at $\alpha<0.01$

Table 7. % Hen day production per treatment group of the Dekalb Single Comb White Leghorn layer pullets supplemented with Avian IgY antibodies and probiotics for 10 weeks.

Week	T1	T2	T3
1	0	0	0
2	0	0	0
3	0.18	0.09	0.26
4	1.49	1.47	2.27
5	7.43	7.92	10.44
6	19.26	22.44	27.40
7	32.76	36.55	43.58
8	49.25	51.54	61.24
9	65.13	67.45	75.29
10	77.92	79.56	84.72

Table 8. % Hen house production per treatment group of the Dekalb Single Comb White Leghorn layer pullets supplemented with Avian IgY antibodies and probiotics for 10 weeks.

Week	T1	T2	T3
1	0	0	0
2	0	0	0
3	0.18	0.09	0.26
4	1.43	1.40	2.20
5	7.02	7.38	9.91
6	18.13	20.83	25.81
7	30.71	33.85	40.83
8	46.06	47.61	57.33
9	60.76	62.13	70.38
10	72.36	73.17	79.11

The supplementation of Avian IgY antibodies and probiotics had a significant effect on the % Hen day production as seen in Table 7. It is shown that T3 had a higher % Hen day production as compared to the other two treatment groups. However, T2 had a higher % Hen day production in comparison to T1. The results of the study in comparison to the ideal % Hen day production for the layer pullets was relatively lower.

The supplementation of Avian IgY antibodies and probiotics had a significant effect on the % Hen house production as seen in Table 7. It is shown in Table 8 that the T3 had a higher % Hen house production than the two other treatment groups. However, T2 had a higher % Hen house production than T1.

Table 9. The total number of eggs for each size and the total weight of eggs produced per treatment group of the Dekalb Single Comb White Leghorn layer pullets supplemented with Avian IgY antibodies and probiotics for 10 weeks.

Egg Size	Treatment 1 (no supplementation)		Treatment 2 (5 days initial supplementation, then twice every week supplementation)		Treatment 3 (5 days initial supplementation, then twice every week supplementation)		Sig.
	Total number	Total Weight (kg)	Total number	Total weight (kg)	Total number	Total weight (kg)	
PL	21,699	1,019.85	23,533	1,106.99	28,017	1,316.80	<0.001*
PW	9,381	440.90	9,670	454.49	10,363	487.06	<0.001*
S	384	18.05	298	14.01	401	18.85	0.0002*
M	67	3.15	87	4.09	97	4.56	0.0615^{ns}
L	14	0.66	5	0.24	12	0.56	0.1152^{ns}
XL	33	1.55	26	1.22	23	1.08	0.3816^{ns}
J	29	1.36	18	0.85	35	1.65	0.0659^{ns}
TOTAL: 1485.46				1581.89		1830.56	

ns = not significant *Significant at α<0.05, **Significant at α<0.01, PL = pullets, PW = peewee, S = small, M = medium, L = large, XL = extra-large, J = jumbo.

Table 10. The feed conversion efficiency per treatment group of the 16-week-old Dekalb Single Comb White Leghorn layer pullets during the 10-week supplementation period.

Week	T1	T2	T3
1	0	0	0
2	0	0	0
3	0.01	0.01	0.021
4	0.08	0.09	0.13
5	0.39	0.47	0.67
6	0.84	0.99	1.16
7	1.23	1.40	1.69
8	1.16	1.21	1.43
9	1.35	1.40	1.55
10	1.52	1.58	1.70

Table 11. The net profit per treatment group of the 16-week-old Dekalb Single Comb White Leghorn layer pullets during the 10-week supplementation period.

	T1 (PhP)	T2 (PhP)	T3 (PhP)
Total egg sales	147,540	156,976.40	181,526.60
Total supplement cost for the treatment group	0	3,510.72	2289.6
Net profit	147,560	153,465.68	179,237
Difference from the control treatment group	N/A	5,905.68	31,677

PhP = Philippines Pesos.

The supplementation of Avian IgY antibodies and probiotics had a significant effect on the total weight of eggs produced as shown in Table 9. The total number of eggs produced per size was totaled per treatment for the whole duration of the experiment and it was multiplied by its corresponding weight. As shown in Table 9, T3 had a higher total weight of eggs produced as compared to the other treatment groups. However, T2 produced a higher total weight of eggs produced over T1. T3 produced 1,830.56 kg of egg, while the T2 produced 1,581.89 kg of egg, and Treatment 1 produced 1,485.46 kg of egg. Statistically, there are significant differences in the production of eggs in Pullets, Peewee, and Small (α=0.05).

The supplementation of Avian IgY antibodies and probiotics had a significant effect on feed conversion efficiency (FCE). As seen on Table 10, the FCE of T3 at week 10 is equal to 1.7 kg of eggs produced per 1 kg of feed, which is higher than two other treatment groups. However, the T2 had a higher FCE at week 10 equal to 1.58 kg of eggs produced per 1 kg feed consumed compared to the T1 with an FCE at week 10 equal to 1.52 kg of eggs per 1 kg feed.

The income over medication cost was computed to determine the added cost per bird of the medication, and to determine the net profit from the supplementation. The additional cost for T2 is 1.84 pesos per bird for the whole supplementation period while T3 has an additional cost of 1.20 pesos per bird for the whole supplementation period. The total egg sales were noted, the additional cost of the supplementation for the whole population per treatment group, net profit, and the total difference of T2 and T3 from the control group. As shown in Table 11, for T2, the total egg sales were 156,976.40 pesos, with a total additional supplementation cost of 3,510.72 pesos, a net profit of 153,465.68 pesos and the total difference from the control group was 5,905.68. For Treatment 3, the total egg sales were

181,526.60 pesos, with a total additional supplementation cost of 2289.60 pesos, a net profit of 179,237 pesos and the total difference from the control group was 31,677 pesos.

Discussion

In this study, the supplementation of Avian IgY antibodies and probiotics which contains *Lactobacillus acidophilus*, *Streptococcus faecium*, *Bifido bacterium longghum*, and *Bacillus thermophilum* had a significant effect on the egg production parameters of the test population which are 16-week-old layer pullets. In the study of Aghaii *et al.* (2010), it was noted that the supplementation of probiotics improved egg production, as well as egg weight, feed-egg ratio, and shell hardness. In this study, the addition of the Avian IgY antibodies along with probiotics in the diet through drinking water significantly increased and helped improve the laying performance of the laying pullets but did not have any significant effect on the growth performance. Probiotics are defined as bio-preparations with live microbial cells that can modulate the balance and activities of gastrointestinal microbiota (Zhang *et al*., 2012). The improvement of egg production performance of laying hens fed Avian IgY antibodies and probiotics may be due to improvement of feed nutrients and improve intestinal health by ameliorating intestinal morphology, altering microbial composition and enhancing microbial community richness.

The effect of the probiotic's supplementation on the average body weight of the pullets was not significant. In a study conducted by Arpasova *et al.* (2012), there were also no observed significant effects of probiotics supplementation on the body weight of the laying hens. The average body weight of the layer pullets should not increase that much as shown in Table 3.

Mortality count was similar across all treatment groups for the whole 10 weeks of the experimentation except on week 4 wherein a strong typhoon hit the farm and leads to a sudden increase in mortality count across all treatment groups for that week. The ideal mortality rate allowed for layers in the whole laying cycle is 2%. In this study, the mortality for 10 weeks across all treatment groups are within the ideal rate. Supplementation of IgY antibodies and probiotics to layer pullets will not have an effect in the growth performance because the focus of the layer pullets is to have better laying performance than growth. The result in the study is similar to the outcome of a study made by Balevi *et al.* (2009), wherein there was also no statistical significance found between the supplementation of probiotics in the diet and mortality count.

As shown in Table 5, there was an earlier onset of laying for the two treatment groups that were given supplementation in comparison to the control group. Probiotics are preparations that can help promote growth by giving a competitive advantage in favour of beneficial bacteria leading to improved nutrient absorption and metabolism (Gutierrez-Fuentes *et al.*, 2013). Avian IgY antibodies which were derived from the egg yolks of hens that are immunized are a more accessible, cheaper but a very good source of polyclonal antibodies (Marcq *et al*., 2013). It was also noted in a study made by Xu *et al.* (2011) that the use of these IgY antibodies did not lead to other adverse effects in comparison to the use of antibiotics. It was also indicated that the Avian IgY antibodies and probiotics from immunized hens can be used to control different poultry diseases such as Salmonellosis, Campylobacteriosis, Infectious Bursal disease, and Newcastle disease (Kimminau *et al.*, 2021; Ahmadi *et al.,* 2021). However, it was also noted that treatment with IgY antibodies mainly concerns in reducing the activity of these infectious agents. The maturity of the gut of the pullets in the T2 and T3 which were supplemented with Avian IgY antibodies and probiotics supplementation was hastened because the supplementation was able to facilitate early colonization of the layer pullets' intestinal tract; this is called competitive exclusion and it leads to the inhibition of the growth and colonization of harmful bacteria (Edens, 2003). This leads to better absorption of the feeds and nutrients given to the layer pullets thus having an earlier onset of laying.

The egg production, % Hen Day production, % Hen house production, total weight of the eggs, and the feed conversion efficiency were all noted in this study. These parameters constitute the overall laying performance of the layer pullets. The said parameters were shown in Tables 5, 6, 7, 8, and 9 respectively. Treatment 3 had higher production numbers across all these parameters among the three treatment groups. The bacteria present in the supplement are known to resist gastric acid secretion and bile salts enzyme effects, they also adhere well and readily colonize the intestinal tract and regulating nutrient digestibility and intestinal morphology, which may be attributed to the alteration of microbial composition and the increase of bacterial diversity (Gibson, 2007; Zhou *et al*., 2021). In the study of Dankowiakowska *et al.* (2013), it was mentioned that probiotics have two major modes of action, first is through competitive exclusion and the second is by boosting the immune system. The use of probiotics and their ability to adhere to the mucosa of the intestines create a natural barrier against potentially harmful pathogens, leading to an improved immune system. These characteristics of the supplement given to the pullets helped the pullets produce more eggs by allowing the layer pullets to absorb more nutrients given through the improved microflora of their gut. However, the T2 had lower production numbers; the reason behind this is that the microflora balance in this treatment group was not optimal due to a more frequent supplementation. The addition of probiotics decreases the colonization of the pathogens and their movement into the layer of pullets' internal organs and eggs and in addition to that this also increases the absorptive surface of the intestines; this leads to better absorption of the nutrients and increased secretions of digestive enzymes which then results to improved utilization of the nutrients delivered in the feed (Dankowiakowska *et al.,* 2013). The results in this study also agree with the study of Kumari *et al.* (2011), who observed that the feed conversion efficiency and the egg production (% Hen Day) were also higher in laying hens that received probiotics supplementation in their diets.

The income over feed, chick, and medication costs were computed and it clearly showed that the supplementation of Avian IgY antibodies and probiotics is economically significant. As shown in table 11, means that the supplementation is economically significant. In addition to that, T3 has a higher net profit than T2 and it can be concluded that the initial supplementation of Avian IgY antibodies and probiotics for 5 days and then twice every 2 weeks for 9 weeks will lead to higher net profit for the poultry farm; therefore, it is more economical to follow this regimen. The regimen followed by T3 is similar to the management practices of the farm; supplements are usually given every 2 weeks and not every week.

Conclusions

Based on the results of this study, the supplementation of Avian IgY antibodies and probiotics in water-soluble powder via drinking water with 5 days initial supplementation of Avian IgY antibodies and probiotics for the first week; then twice every 2 weeks for 9 weeks has the greatest effects on the age in days when 50% of the total population per treatment laid their first eggs, egg production, % Hen Day production, % Hen housed production, the total weight of eggs produced, and feed conversion efficiency. The supplementation also proved to be economical and will lead to higher net profit for the poultry farm. On the other hand, it has no significant effects on the mortality count and the average body weight of the layer pullets.

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