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EFFICIENCY OF USING PROBIOTIC ELAC-GROW AND HAN-PROWAY IN LAYING-EGG HENS

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SUMMARY

Industrial chicken farm has to face many challenges such as epidermis and environmental pollution. Antibiotics and synthetic antimicrobial agents are routinely used to alleviate stress as well as to elevate growth and feed efficiency. However, using antibiotic had many negative effects, especially antibiotic resistance; therefore, probiotic products are a bio-secure solution being popularly applied in livestock husbandry. The study was carried out on the two probiotics Elac-grow, a diet supplement, and Han-proway, a waste treatment product, in industrial egg-laying hens. There were two lodgings (test and control) in these experiments; each had 4000 23-week-old hens of Hyline breed. Two probiotics were applied to the test and none to the control. The results showed that there was not significantly difference of egg yield between the two groups, although, the test lodging had higher egg yield growing ratio (67.48%) than the control one (65.73%). Egg quality in the experimental lodging was also significantly improved in the damaged egg ratio, the egg weight and the egg yolk weight after using Elac-grow. The efficiency of using Elac-grow and Han-proway was also shown through the concentration of ammonia emission in animal lodging. The ammonia value of the test lodging was maximum 5 ppm and remained stable at this point, lower than of the control. This efficiency was shown significantly in the microbial fluctuation analysis, when the total bacteria and coliform counts were reduced in the test lodging sequentially above $1x10^8$ CFU bacteria/g and above $1x10^7$ CFU coliform/g, the control had uptrend sequentially above $2x10^9$ CFU bacteria/g and above $1x10^8$ CFU coliform/g.

Keyword: Ammonia value, Elac-grow, egg yield, egg quality, Han-proway, probiotics

INTRODUCTION

Probiotics are defined as live microbial feed supplements that benefit host animals by improving their intestinal microbial balance (AFRC, 1989). The addition of probiotics to diets has been demonstrated to improve growth performance and the feed conversion ratio in broilers (Nahashon *et al*., 1996a), as well as their egg mass, egg weight, egg size in layers, (Nahashon *et al*., 1996b), feed consumption (Nahashon *et al*., 1994) and egg-specific gravity (Nahashon *et al*., 1996b) in layers.

Many researches showed that the mode of action of probiotics in poultry included maintaining normal intestinal micro flora by competitive exclusion and antagonism, altering metabolism by increasing digestive enzyme activity and decreasing bacterial enzyme activity and ammonia production, and improving feed intake and digestion neutralizing enterotoxins (Jin *et al*., 1997). *Lactobacillus* spp. and *Enterococcus* spp. are the most commonly used species in the production of probiotics. Enterotoxin produced by pathogenic bacteria can be neutralized by the substances produced by these species. Zhang *et al* (2019) showed that the dietary supplementation with *Enterococcus faecium* did not affect the average daily egg weight, cracked egg rate, mortality and egg quality. However, *Enterococcus faecium* supplementation caused a significantly increased laying rate and decreased feed/egg ratio. The differences in caecal microbiota between test group and the control were significant (Zhang *et al*., 2019).

Moreover, under such circumstances, antibiotics and synthetic antimicrobial agents are often used to alleviate stress and to elevate growth and feed efficiency. However, injudicious and persistent sub-therapeutic antibiotics addition in poultry feed have become undesirable due to their residues in meat products and development of antibiotic-resistant bacterial population (Jin *et al*., 1997). In Europe, the use of antibiotics as growth promoting agents in poultry has been banned. Due to several negative effects of antibiotics, these have been gradually replaced by probiotics (AFRC, 1989), (Patterson and Burkholder, 2003; Dhama *et al*., 2011).

On the other hand, breeders were also concerned about the waste treatment, which directly affect hens and people. The use of probiotic additives has lowered the quantity of nitrogen in waste effluent, which potentially represents a gain in feed efficiency and reduced nitrogen requirements in diet formulations, resulting in a reduction of leached nitrogen in the farm and the surrounding environment (Applegate *et al*., 2010).

With the purpose of providing bio-secure livestock solution, two probiotics were produced by Hanvet Pharmaceutical Limited Company: Elac-grow for hen’s diets and Han-proway for waste treatment. The aim of the present study was to investigate the effects of these two commercial probiotics Elac-grow for feed diet and Han-proway for waste treatment on, egg quantity, egg quality, emission evaluation and microbial fluctuation in laying hens.

MATERIALS AND METHODS

Commercial probiotics

Elac-grow for feeding and Han-proway for waste treatment are produced by Hanvet Pharmaceutical Limited Company. For this experiment, 1 kg of Elac-grow was used for 1000 kg feed, 3 days per week, 1 kg of Han-proway was used for 80-100 m^2 composite, periodically every 7-10 days. Components of 1 kg Elac-grow include *Enterococcus faecium* ($7x10^{10}$ CFU), mix of vitamin (A, D2, E, B2, B6, B12, K, C), $MgSO_4$, $CuSO_4$, $ZnSO_4$, calcium, DL-methionine, niacinamide, citric acid, lysine. Components of 1 kg Han-proway include *Bacillus licheniformis* ($2.7x10^9$ CFU), *Bacillus megaterium* ($2.5x10^9$ CFU), *Lactobacillus acidophilus* ($3x10^9$ CFU).

Laying hens and farm facilities

The experiment was performed at Hoa Phat farm, a laying hen farm in Dong Anh, Hanoi, Vietnam. It is an area of 500 m^2 with a system of eight exhaust fans in the rear and four rows of double nest. Two animal lodgings were set up for this experiment; each of them had 4000 laying hens. All laying hens belonged to the same Hyline brown variety at the age of 23 weeks.

Experimental design

Experiment lodging: using probiotic Elac-grow for feed and Han-proway for waste treatment; control lodging: did not use probiotic for feed and for waste treatment.

Effect of using probiotic in diet to egg production

All of the eggs were collected and weighted individually to determine the total number of the egg and the egg weight. Using these values, egg production, birth rate and egg weight were calculated.

Egg yield (egg) = The total number of egg

$$\text{Birth rate (\%)} = \frac{\textit{the number of eggs} \times 100}{\textit{the number of hens}}$$

$$\text{Egg weight (g/egg)} = \frac{\textit{the sumery of egg weight (gr)}}{\textit{the number of eggs (egg)}}$$

Egg quality was determined. Thirty eggs were collected randomly from each lodging. Each egg was estimated, the york weighted, york color checked to determine egg quality

Effect of using probiotic for waste treatment

Gas detector of Bosean BH-4S (China) was used to test the amount of ammonia in the lodging. Ammonia level measurement was carried out during the day from 11 am to 12 am to get the highest ammonia level caused by evaporation that occurred due to heat.

The fluctuation of microbial in the waste was analyzed in the laboratory

Compost analysis was examined for Coliforms and total bacteria by viable cell numeration method. Coliforms were analyzed on Mackonkey agar (Merck), total bacteria were analyzed on Tryptic Soy Agar (TSA, Merck). Compost samples were collected from the top layer of the middle lodging from 16 pm to 17 pm.

Statistical analysis

All results were statistically analyzed by Excel 2010.

RESULTS AND DICUSSION

Effect of using probiotic in diet

Egg laying rate and egg yield

Nahashon *et al*. (1994a,b, 1996b) and Tortuero and Fernandez (1995) reported that there were significant increases in egg production of laying hens by using various levels of probiotic supplementation. Our result showed the same result that there was not a significant increase in the egg yield between test and control. However, based on Figure 1, although the birth rate of the control lodging was higher than that of the test lodging, the speed of growing egg yield in the test lodging was higher than that in the control lodging after one week of using probiotics. The egg laying rate in test lodging increased from 19.7 to 87.18%, when this value in control lodging increased more slowly from 23.39 to 89.12%. Therefore, egg yield growing rate of the test was 67.42% higher than 65.73% in the control.

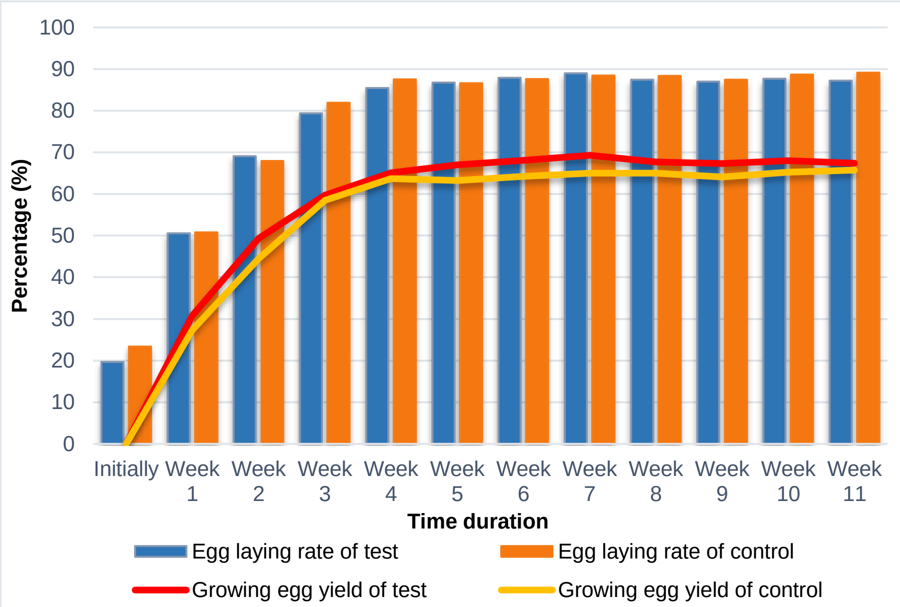

Figure 1. Comparison of the egg laying rate and the growing egg yield between test lodging and control lodging.

Quality of egg

The damaged egg ratio was based on the hardness of eggshell through calcium supplementation in the diet. In spite of having higher damaged egg rate initially, this rate of the test lodging was improved immediately after adding Elac-grow in the diets for one week and maintained a lower value than the control lodging throughout experiment (Figure 2). After 11 weeks, the damaged egg rate of the test lodging was 0.15% lower than 0.23% of the control (P<0.05). Balevi *et al* (2001) reported that the damaged egg ratio was lower (0.76%) than the control (1.42%) with the 500 mg/kg probiotic supplementation into laying hens' diets (p<0.05) (Balevi *et al*. 2001). Kurtoglu *et al*. (2003) also reported similar results (Kurtoglu *et al*., 2004).

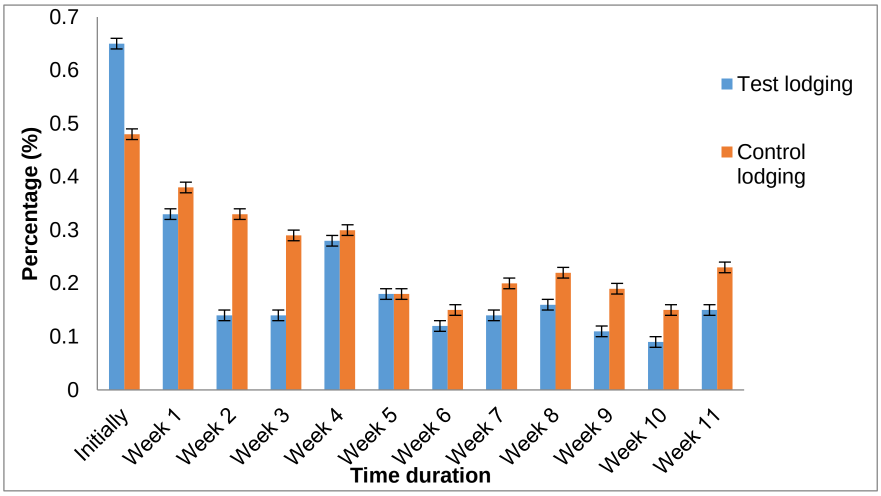

Figure 2. The damaged egg ratio of test lodging and control lodging.

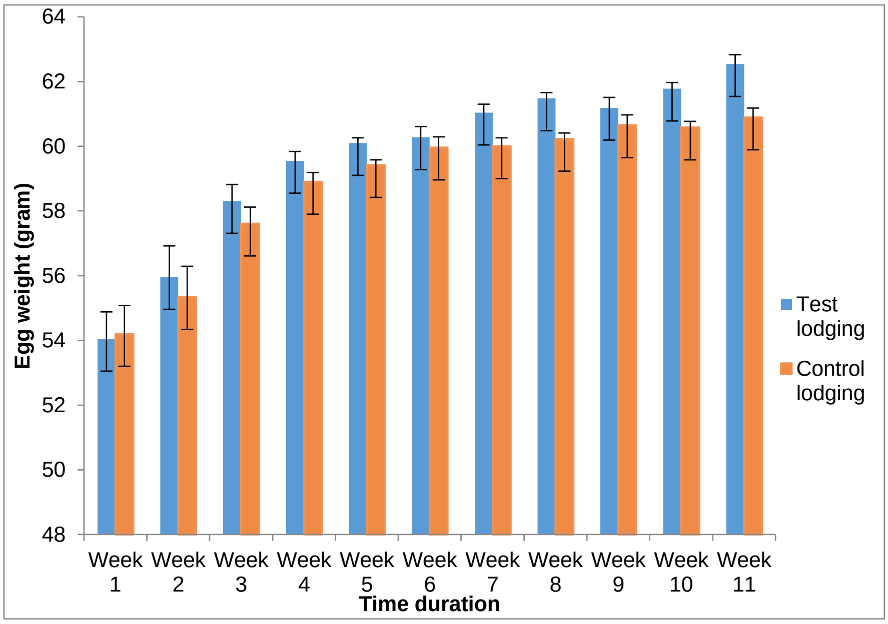

Figure 3. The average egg weight value of test lodging and control lodging.

The egg weight of test lodging was also improved after two weeks of using Elac-grow. This value of test lodging increased 8.25 units from 54.05 to 62.54 g, when the control lodging only increased 7.27 units from 54.2 to 60.89 g ($P<0.05$). This was similar to the result in the publication of Tortuero and Fernandez (1995) and Nahashon *et al*. (1994b), which observed that significant increases in egg weight were obtained from probiotic addition (Tortuero, Fernández 1995; Nahashon *et al*., 1994).

The egg yolk weight of the test lodging was higher than that of the control. In the test lodging, the egg yolk weight increased from 11.2 to 14.49 g, this value raised from 11.5 to 14.42 g in the control one ($P<0.05$) (Figure 4).

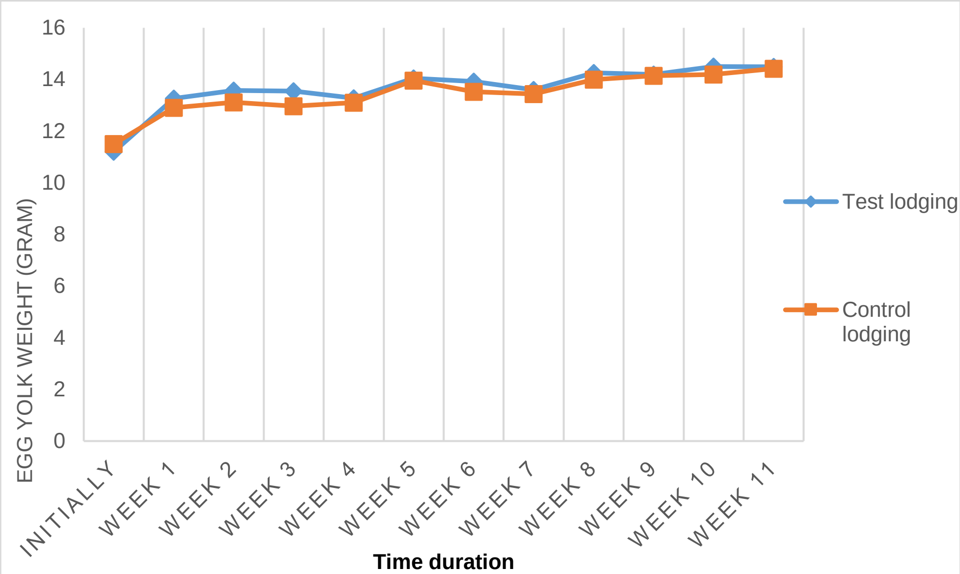

Figure 4. The egg yolk weight of test lodging and control lodging.

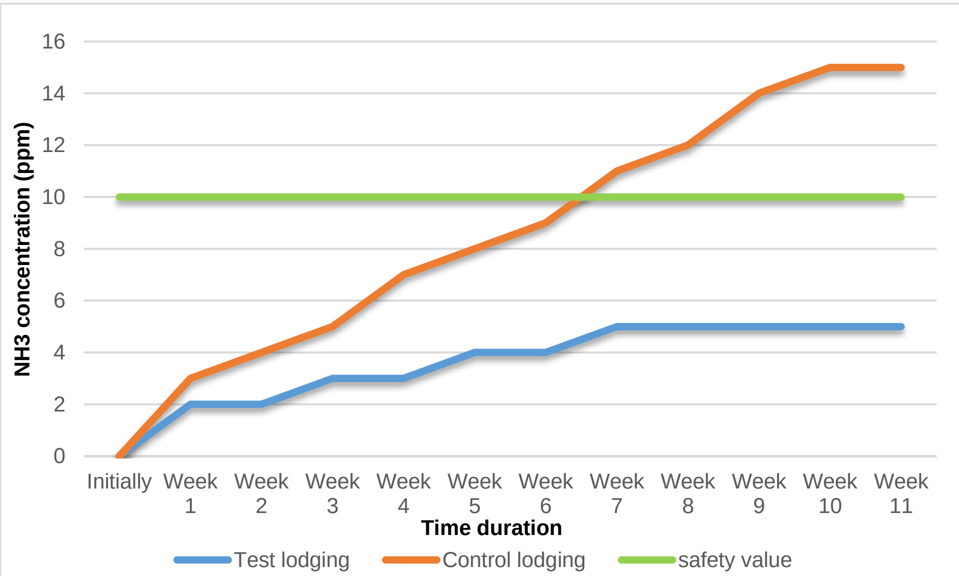

Figure 5. Comparison of NH_3 concentration in the test lodging and in the control lodging.

Evaluate emissions in the lodging

Hens' health, egg quantity and quality are not only affected by the diets, but also by the surrounding environment. Ammonia is a gas present in the atmosphere of every poultry house. It results from the chemical decomposition of uric acid in droppings by certain bacteria in the litter (TahseenAziz, 2010). According to National Technical Regulation poultry farms were considered biosecurity when ammonia concentration in the lodging was less than 10 ppm (QCVN01-15: 2010/BNNPTNT). Figure 5 showed that by using the probiotic Han-proway for waste treatment, the test lodging had lower ammonia value of 5 ppm than the control of 15 ppm and the safety value of 10 ppm. Research of Song *et al* in 2012 showed that poultry manure is one of the major sources of nitrogen pollution (Song *et al*., 2012), of which ammonia is a major aerial pollutant, with adverse effects on the production of broilers (Miles *et al*., 2004; Wathes and Kristensen, 2000). Park et at showed that laying hens fed the probiotic *Enterococcus faecium* supplementations had lower ammonia emission than those fed the control treatment (Park *et al*., 2016).

The fluctuation of microbial in the waste

The fluctuation of microbial in the waste showed significant difference after using probiotic solution, especially in coliform density (Table 1). When the total bacteria and coliform density of test lodging decreased and stabilized at more than 1×10^8 CFU bacteria/g and more than $1x10^7$ CFU coliform/g, these value in the control increased rapidly with more than $2x10^9$ CFU bacteria/g and more than $1x10^8$ CFU coliform/g. This result was the same with the research of Park *et al* (2016), coliform counts were reduced with the probiotic treatment as compared with the control treatment ($P < 0.05$) (Park *et al*., 2016).

Table 1. Fluctuation of microbial density and coliform density.

Time duration	Total microbial density (CFU/g)		Coliform density (CFU/g)	
	Test lodging	**Control lodging**	**Test lodging**	**Control lodging**
Initially	$2.12 \pm 0.13\times10^8$	$3.12 \pm 0.15\times10^8$	$1.12 \pm 0.19\times10^8$	$1.67 \pm 0.15\times10^8$
Week 1	$2.05 \pm 0.21\times10^8$	$5.23 \pm 0.17\times10^8$	$8.95 \pm 0.28\times10^7$	$1.55 \pm 0.25\times10^8$
Week 2	$1.98 \pm 0.19\times10^8$	$5.5 \pm 0.15\times10^8$	$6.65 \pm 0.24\times10^7$	$1.75 \pm 0.18\times10^8$
Week 3	$1.85 \pm 0.22\times10^8$	$1.35 \pm 0.2\times10^9$	$4.25 \pm 0.18\times10^7$	$1.23 \pm 0.24\times10^8$
Week 4	$1.92 \pm 0.18\times10^8$	$1.22 \pm 0.14\times10^9$	$3.45 \pm 0.12\times10^7$	$1.35 \pm 0.21\times10^8$
Week 5	$1.76 \pm 0.25\times10^8$	$1.45 \pm 0.27\times10^9$	$2.25 \pm 0.22\times10^7$	$1.74 \pm 0.19\times10^8$
Week 6	$1.5 \pm 0.17\times10^8$	$1.55 \pm 0.13\times10^9$	$2.12 \pm 0.18\times10^7$	$1.67 \pm 0.21\times10^8$
Week 7	$1.56 \pm 0.13\times10^8$	$2.12 \pm 0.17\times10^9$	$1.65 \pm 0.2\times10^7$	$1.98 \pm 0.19\times10^8$
Week 8	$1.72 \pm 0.21\times10^8$	$2.25 \pm 0.25\times10^9$	$1.22 \pm 0.22\times10^7$	$1.68 \pm 0.27\times10^8$
Week 9	$1.32 \pm 0.19\times10^8$	$2.5 \pm 0.21\times10^9$	$1.32 \pm 0.27\times10^7$	$1.74 \pm 0.25\times10^8$
Week 10	$1.35 \pm 0.21\times10^8$	$2.45 \pm 0.25\times10^9$	$1.25 \pm 0.23\times10^7$	$1.82 \pm 0.27\times10^8$
Week 11	$1.22 \pm 0.15\times10^8$	$2.55 \pm 0.2\times10^9$	$1.05 \pm 0.17\times10^7$	$1.92 \pm 0.21\times10^8$

CONCLUSION

We could realize a slight improvement of birth rate and growing egg yield in test group, however, further investigation should be carried out to address this effect, the test lodging had higher egg yield growing ratio of 67.48% than the control one of 65.73%. The quality of egg in the experimental lodging was also significantly improved in the damaged egg ratio, the egg weight and the egg yolk weight after using probiotic Elac-grow. The efficiency of using probiotic Elac-grow and Han-proway was also shown through the concentration of ammonia emission in the animal lodging. The ammonia value of the test lodging was maximum 5 ppm and remained stable at this point, which was lower than the control. This efficiency was shown significantly in the microbial fluctuation analysis.

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