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EFFECT OF AQUA BIOTA-P SUPPLEMENTATION ON EGG QUALITY PARAMETERS OF COMMERCIAL LAYER HENS

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ABSTRACT: The effect of Aqua Biota-P supplementation on egg quality of commercial layer hens was assessed. A total of 300 Hy-Line Brown layer birds of 33 weeks age were used for the study. Treatment and control groups had 150 hens each that were further divided into three replicates of 50 birds each. Layers in treatment group were fed Aqua Biota-P @ 1g/kg feed for 10 days and 0.4g/kg feed for the next 32 days. The egg variables; egg weight, eggshell thickness, albumen height, shell discoloration, egg production and ammonia gas emission from the urine and feces were assessed. A t-test was used to determine difference between the means of control and treatment groups. Correlation test was performed to find the association between Haugh Unit and albumen height. At the end of 42 days trial period, the findings revealed highly significant ($p < 0.003$) differences between groups in all the test parameters as a result of feeding Aqua Biota-P. The most significant finding obtained was in improved Haugh Index indicating better-quality egg with higher protein content. Further, a 6% increase in egg production was observed in treatment group. Also, the bedding material in the treatment group had lesser amount of odour and maintained dryness. Thus, the study concludes that Aqua Biota-P supplementation in commercial layers had positive impact on egg quality parameters and health of the birds.

Keywords: Aqua Biota-P; commercial layers; egg quality; health

1. INTRODUCTION

Antibiotics have been used to maintain animal health, promote growth and improve production efficiency for more than 50 years. However, as early as the 1950s, researchers identified concerns on the development of antibiotic resistance such as use of streptomycin and tetracycline in turkeys and broilers respectively (Abd El-Hack et al. 2020). To offset the spread of antibiotic resistance, newer approaches; reduction in antibiotics usage and adoption of probiotics as an alternative to antibiotics were advocated (Ouwehand 2016)

Excessive use of antibiotics in farmed animals results in the development of bacterial strains with antibiotic resistance. The spectrum may range from diarrhea without mucosal abnormality to pseudomembranous colitis. The latter is a severe form of antibiotic-associated diarrhea caused by Clostridium difficile and cytotoxic strains which is expected to have emerged from use of antibiotics (Kechagia et al. 2013). Moreover, excessive accumulation of these resistant bacteria in farmed animals especially in poultry decreases micro biota efficacy to absorb nutrients like calcium, a key compound for egg shell formation resulting in production of fragile eggs.

Thapa (2018) recorded a high prevalence of anti-microbial resistance (AMR) to commonly used antibiotics in Bhutanese livestock. Although many factors including egg augment the conditions mentioned, poultry product like egg plays vital role as a dietary supplement. Egg, apart from being affordable source of protein, also contains harmful components like cholesterol. The relationship between blood cholesterol and heart disease is well-established, with the lowering of serum low-density lipoprotein (LDL) cholesterol being the primary target of preventive therapy. Epidemiological studies suggest lower risk of heart disease with higher concentrations of high-density lipoprotein (HDL) cholesterol (Blesso 2018).

Probiotics are defined as live microorganisms which can confer a health benefit for the host when administered in regular and appropriate quantities (Chaucheyras-Durand & Durand 2010). Also, it is defined as microbial food supplements

which beneficially affect the host animal by improving its intestinal microbial balance, improves feed conversion for the target species, reduce morbidity or mortality and benefit the consumer through improved product quality (Musa et al. 2009).Microbiota plays a vital role in well-being of both the animal and humans through improvement of metabolism and immune system (Chaucheyras-Durand & Durand 2010). In poultry, it influences the productivity and the quality of products (egg and meat). Gut microbiota profile influences immune homeostasis and inflammatory state, which contribute to the animal's health. Chicken health and productivity is also dependent on the capacity to extract and absorb nutrients and sources of energy from feeds, in which the gut microbiota provides an extensive array of enzymes and substrates. Gut microbiota qualitative and quantitative profile has a key role in the efficiency of energy extraction from feed. Microbiota also produces short chain volatile fatty acids that modulate the metabolism in chicken. Foods containing probiotic microbes for human consumption had been marketed in Japan since 1920s and the first bacteria used were *Lactobacillus acidophilus* and *Lactobacillus casei* components of fermented milk products (Musa et al. 2009).

Aqua Biota-P is a probiotic, developed by INNOVATION LABO, targeted for modulation of chicken gut microbiota. It contains lactobacillus strain LBM-7 selected amongst 200 varieties for its efficacy and resistance to acidic stomach environment. Feeding Aqua Biota-P in poultry and evaluating the benefits of the microbiota can assist in maintaining the well-being of birds and production of quality products, which indirectly improves the human health through consumption of the quality products (egg, meat). These are considered as quality products since they are produced without the use of antibiotics. There are research findings on probiotic supplementation in poultry diet and its effect on egg production performance and egg quality. However, until date, very limited scientific information is documented on Aqua Biota- P supplementation in feed and its effect on egg quality and health performance of commercial layer hen which warranted this research.

There are many probiotics available for use in agriculture including poultry industry. One of the main brands for everyday use is EM-1. However, there is information gap on the use and effect of EM on egg quality and laying performance of chickens in the Bhutanese context.

2.MATERIALS AND METHOD

2.1 Study Area and sampling

The study was carried out at the commercial layer farm located at $26^0 52' 57"$ N, $90^0 14' 39"$ E of Bijay Rai, Phuntshogang village, Getemkha chiwog, Gakidling gewog by the National Poultry Research and Development Centre (NPRDC) under Sarpang Dzongkhag. A total of 300 Hy-Line Brown strain of commercial layer birds were randomly subjected to the study. 150 hens each was assigned as treatment and control groups with 3 replicates consisting of 50 birds each in both the groups. 150 hens in treatment group were subjected to Aqua Biota-P feeding for a period of 42 days. The age of the hen were 33 weeks at the commencement of this study.

2.2. Housing and stocking environment

The hens in both the groups were housed in a modern poultry housing system following international standard stocking density of 8 birds/m^2. Galvanized wire mesh was used for partition within the replicas in both the groups. Approximately 1.3 cm thickness of bedding material (saw dust) was provided in both the groups for comfort of the birds.

2.3. Feed and Feedings

Commercial layer mash feed, manufactured by Karma Feeds was used for feeding the birds. Karm Feeds was fed to the hens in the control group as per the international standard for the breed. Birds in treatment group were fed with the mixture of aqua biota-p @ 1g of aqua biota-P in a 1000 gm of feed for 10 days and 0.4g of Aqua Biota-P in a 1000 gm of feed for the next 32 days. Fresh clean water was provided ad-libitum to both the groups during the study period.

2.4. Data collection and analysis

Every week, 24 eggs from both treatment and control groups were randomly sampled and evaluated against the test parameters as per the guide provided by INNOVATION LABO. Eggs

were evaluated against the following parameters: egg shell thickness, egg shell discolouration, albumen height and egg weight. Daily egg production was recorded in the recording sheet and later transferred in Excel Sheet. Egg Shell thickness was measured using standard Vernier caliper. Each egg shell thickness was measured at three places and averaged. Albumen height was measured in petri dish with stainless steel millimeter scale. Egg weight was measured with a digital weighing balance from AN ISO: 9001:2008 certified company. Weekly collected data were computed in excel sheet and analyzed in Statistical Package for Social Science version 26 (SPSS 26) for obtaining the result. For Haugh Unit (HU), a t-test was used to determine if there is a significant difference between the means of two groups (treatment and control). The same test was used to find the presence or absence of differences between groups for eggs shell thickness, egg production and, egg weight. Correlation test was used to evaluate the association between the HU and albumen height.

3. RESULTS AND DISCUSSION

After completion of 42 days' feeding trial of Aqua Biota-P in commercial layer hens as per experimental design, either significant differences or at least mean differences throughout the test parameters like HU, eggs shell thickness, egg shell discoloration, and egg production for the samples were observed. Although there was no statistically significant difference, upon physical examination, the environmental factors like bedding materials in the treatment group had less odour and maintained dryness. This indicates improved environmental condition of the shed and thus the improved welfare and health of the layers when supplemented with Aqua-biota-P in the feed.

3.1 HU and albumen thickness

HU is a measure of egg protein quality based on the height of its egg white (albumen) and its weight (Eisen et al. 1962). The height of the thick albumen surrounding the yolk, combined with the egg weight, determines the HU score. The higher the score, the better the egg quality. To calculate the HU, the formula used was:

$$HU = 100 \text{ Log } (h - 1.7\ w^{0.37} + 7.6)$$

Where:

HU = Haugh unit
h = observed height of the albumen in millimeter
w = weight of egg in grams

Haugh Index (HI): As per United States Department of Agriculture (USDA), description of egg white and corresponding HU are as follows:

AA:	72 or more
A:	71 - 60
B:	59 - 31
C:	30 or less

The albumen thickness of the randomly sampled eggs was measured and HU calculated using formula mentioned above.

HU in treatment group is higher than the control group which means the measure of HU between two treatments had differences. The mean difference from t-test result reveals that the HU for the treatment group is 95.33 units (equivalent to AA grade egg) and control group is 53.50 units (equivalent to B grade egg). Correspondingly the equal variance assumed for t-test ($t = 3.832$, $p = .003$) indicated a statistically significant difference between the groups. HU is directly proportional to the albumen thickness of an egg. As the albumen thickness increases the HU too increases. To determine the relation between the two variables, correlation test was performed and test results indicate that the correlation is highly significant at 99% confidence interval.

3.2 Egg shell thickness

The average mean eggshell thickness in treatment group was found 0.03 mm thicker than in control. The eggshell thickness recorded was 0.60 mm and 0.57 mm for the treatment and control group, respectively. The improvement of egg shell color by feeding Aqua biota P is not significant in commercial layer birds at 33-39 weeks of age. However, mean difference of 0.33 mm on egg shell thickness between treatment and control groups was obtained in this trial.

3.3 Egg weight

The mean egg weight in treatment and control group were similar without any significant

differences. This indicate that there was no effect of Aqua Biota-P supplementation on the egg weight. The mean egg weights of treatment and control groups were 63.5 g and 63.6 g respectively.

3.4 Egg production

Total egg production showed a significant difference (p=.000). This means that there was an influence of Aqua Biota-P supplementation on the egg production percentage. The weekly egg production percentages were 88% (≃264 nos.) and 82% (≃245 nos.) for treatment and control group, respectively. An increase in egg production by 6 % is attributed to Aqua Biota-P supplementation alone since all other confounding variables remain the same.

3. CONCLUSIONS & RECOMMENDATION

The Aqua Biota P supplementation in commercial layer birds had a significant effect on the egg quality across different egg quality parameters, viz, eggshell thickness, albumen thickness (height of egg white) and egg production. The most significant findings were obtained in HU indicating better-quality egg with higher protein content since HU/HI is directly proportional to the protein content. Total egg production also was significantly higher in the treatment group. Dryness of bedding material with lesser ammonia production too was observed in the treatment group. Aqua Biota P is available in dry substrate form and do not mix uniformly with pellet form of feed. Liquid carrier substrate would be more suitable in poultry industry as pellet form is the most preferred commercial feed type. Thus, this study concludes that the Aqua Biota-P supplementation in commercial layer promotes improved egg quality and animal welfare (dryness of bedding and reduced odour). However, more comparative studies are required to study the efficacy of Aqua Biota P vis-à-vis other commercially available probiotics in liquid form.

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