Bangladesh Journal of Animal Science 53 (2): 31-39, 2024 https://doi.org/10.3329/bjas.v53i2.74538

Bangladesh Journal of Animal Science
Journal homepage: http://www.banglajol.info/index.php/BJAS

Effect of butyrine glycerides on the performance metrics and egg quality in laying pullets

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ARTICLE INFO

Article history:
Received: 23 February 2024
Revised: 7 March 2024
Accepted: 23 June 2024
Published: 30 June 2024

Keywords:
Butyric acid, egg quality, performance, pullets.

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ISSN: 0003-3588

OPEN ACCESS

ABSTRACT

The chicken feed industry is very much interested in creating innovative solutions to address the issue of safer animal products due to the increasing customer demand and the prohibition on utilizing antibiotic growth boosters. Organic acids, especially butyric acid, when added to chicken diets, have several beneficial effects on health, productivity, and egg quality. These factors may lead to their substitution in animal production systems for antibiotics. The study was determined the effects of butyrine glycerides (BAG) supplementation on various aspects of egg production and egg quality in laying pullets. Three dietary treatments were randomly assigned to 48 NovoGen Brown laying pullets at 24 weeks of age. For eighteen weeks, the chickens were kept in a three-tiered pyramid-style cage. The chickens in the control group (T1) were fed a basal diet; in groups T2 and T3, the basal diet was supplemented with 0.033% and 0.066% butyrine glycerides, respectively. Throughout the 12 weeks trial, measurements were made of the quantity and weight of eggs laid, feed conversion, and egg quality. Dietary supplementation of butyrine glycerides contributed to a significantly ($p<0.001$) increased in the egg production, egg weight and egg mass in laying pullets. Similarly, egg quality parameters did not affect significantly except shell percentage, albumen index and Haugh unit at 28th weeks of supplementation. Taken altogether, it is said that BAG positively impacted the laying performances and egg quality of laying pullets.

Introduction

One of the millimolar amounts of short-chain fatty acids (SCFAs) produced in the avian cecum is butyric acid, where microbial fermentation of unabsorbed starch primarily occurs (Liu et al., 2017). Because butyric acid is quickly absorbed in the upper gastrointestinal tract (GIT) when it is unprotected, it appears that protection is necessary for the small intestine to benefit (Kaczmarek et al., 2016; Elnesr et al., 2020; Silva et al., 2020). Following the restrictions on the use of in-feed antibiotic growth promoters, the poultry industry has concentrated on developing innovative strategies to enhance performance by increasing energy and nutrient intake while maintaining and maybe even enhancing the health of the birds. There has been a growing amount of research done on the application of organic acids to enhance performance metrics in layers and broilers. Because of the supply, nutrition, and environment of organic acids, such as butyric acid and its salts, growth outcomes, egg production, and egg quality have all shown positive benefits

How to Cite

B Dey, PK Goswami, QAH Roddur, N Jahan, S Koly and AR Asif (2024). Effects of butyrine glycerides on performance metrics and egg quality in laying pullets. *Bangladesh Journal of Animal Science* 53 (2): 31 – 39. https://doi.org/10.3329/bjas.v53i2.74538

(Elnesr et al., 2019; Maty and Hassan, 2020). By giving villi a carbon source to grow, encouraging the expansion of *lactobacilli* and *Bifidobacteria*, and lowering luminal pH, organic acids like butyric acid help to enhance gut health by reducing the growth of dangerous bacteria like *Salmonella*, *Clostridium*, and *Escherichia coli.* It is hypothesized that better digestive system wellness will enable higher absorption, which will raise nutrient and energy consumption in chickens and enhance performance (Qaisrani et al., 2015; Maty and Hassan, 2020).

Important economic variables in layer farming are the amount and quality of eggs produced. Greater eggshell strength without sacrificing ideal egg size is a sign of higher-quality eggs. Calcium is taken up from the small intestine and integrated into the eggshell and bone, making it an important component of the meal for layer diets (Saunders-Blades et al., 2009; Makled et al., 2019). Eggshell thickness and breaking strength decrease with ageing hens due to a decrease in their ability to absorb nutrients, particularly calcium. Because of broken eggs, this results in higher financial losses (Molnár et al., 2018; Maty and Hassan, 2020). Organic acids improve the absorption of minerals from the colon by lowering the pH of digesta and inhibiting the formation of molecules between calcium and phytate (Rafacz-Livingston et al., 2005). Supplementing with butyric acid and its salts, such as sodium butyrate, has been shown to raise serum levels of phosphorus and calcium and magnesium levels (Kamal and Ragaa, 2014).

When butyric acid was supplied in a coated form, like encapsulation, its effectiveness increased, indicating that the GIT benefited from this protection (Kaczmarek et al., 2016; Elnesr et al., 2020). Results from earlier research were inconsistent, which could have been caused by variables like age, nutrition, feed composition, experimental setup, flock health, butyric acid source, and inclusion rate (Kaczmarek et al., 2016). Though Levy et al. (2015) found no appreciable difference in villi height between encapsulated butyric acid and controls, Kaczmarek et al. (2016) found that in broilers, various doses of protected butyrate significantly enhanced both villi height and apparent metabolizable energy (AME). However, there is a significant amount of dissimilarity and inconsistency in the results reported across different studies. This inconsistency highlights the need for more studies to clarify the understanding of the effect of BAG on performance matrix, egg quality along with their gut health. This study was done with a goal to assess how BAG affects laying pullets' performance metrics and egg quality.

Materials and Methods

Experimental birds

Forty-eight 24-week-old NovoGen-Brown laying hens were obtained from the BAU Poultry Farm of Bangladesh Agricultural University, located in Mymensingh, Bangladesh. The birds were kept in a layer cage designed like a three-tier pyramid at the BAU Poultry Farm, where the experiment was carried out. Each pen had a square that was the same size and held two birds. The cages were thoroughly cleaned, rinsed, and disinfected before the experimental birds were arranged. They were then left to dry. In addition, a thermometer with a humidity gauge were utilized to keep the relative humidity and temperature of the residence at their respective levels. The experimental birds were divided into three dietary treatment groups randomly, with four replications per treatment and maintained four birds per replication.

Birds' husbandry

A 20 by 20-foot open-sided shed-style house served as the site of the feeding study. The shed's ceiling was eight feet above the ground. Electric ceiling fans were employed for ventilation even though the house had natural ventilation. The birds were given unlimited access to potable water and two meals a day, in the morning and the afternoon. Ten days were allotted to the birds for feed modification and acclimatization; during this time, no study data were collected. While the laying stage is underway, a total of sixteen hours of light (a combination of artificial illumination and natural daylight) were delivered. Artificial light was supplied to maintain the right amount of lighting during overcast weather or when natural light was insufficient. A constant 20–30 lux light intensity was maintained.

Preparation of experimental diet

The NRC's (1994) recommendations were the basis for the diet developed for NovoGen-Brown laying hens. Each feed item was purchased from the local market and manually prepared. After carefully mixing, three equal portions of the diet were fed. Diet was considered as independent variable where there were three treatments (T1=control; T2= 0.033% butyrine glycerides; T3=0.066% butyrine glycerides). In this investigation, butyrine glycerides were used in the form of Tributyrin, a glycerol ester of butyric acid with silica, which was developed by the Dutch company FARMELCO.

Performance variables

Every day, weights and production rates of eggs were recorded. Weighing the collected eggs allowed us to record their weights according to cage. Feed consumption and laying hen weight of the body was noted at the start and end of the trial. Weekly mortality was recorded to calculate feed conversion ratio (FCR) and average daily feed intake (ADFI).

Record Keeping

Every day and every week, data from the experimental trial were gathered. Prior to the experiment commencing, the initial body weight was noted, and at its conclusion, the ultimate weight of the body was noted. Two daily readings of the temperature and humidity were made starting at the start of the feeding trial. Following the first period of acclimatization, laying performance metrics, including egg weight and production, were measured every day in the afternoon. At the conclusion of the feeding trial, figures such as average daily feed intake, egg mass yield, feed conversion ratio, etc. were computed. Throughout the course of the experiment, four-week intervals were maintained between the collection of eggs.

Egg quality measurements

The egg quality indices were determined using collected eggs. Of 48 eggs, 12 were used to measure the weight of the egg and the egg shell, while the remaining 12 eggs were used to measure both the internal (albumen and yolk index) and external egg quality. A quasistatic compression device was used to crack each egg after it had been individually weighed to determine the eggshell breaking strength. Three points on the eggshell the middle, top, and bottom ends were tested for thickness using a micrometer screw gauge. Using a tripod micrometer, the albumen's height (average of four places) was determined to be midway between the thick albumen's edge and yolk (Jahanian and Rasouli, 2014b). The Haugh unit was calculated using the following formula: HU¼100 log (Hþ7.571.7W0.37), where W is the provided egg's weight (g) and H is the albumen's mean height (mm) (Silversides, 1994). The yolk index was calculated by dividing the height and diameter of the yolk. The Rochi yolk color fan was used to score yolk color graphically.

Statistical analysis

In a completely randomized design (CRD), data were submitted to analysis of variance (ANOVA) using the Statistical Computer Package Software (SAS, 2009). Duncan's Multiple Range Test (DMRT) was used to compare the means, and p-values of less than 0.05 were regarded as significant.

Results and Discussion

Data were analyzed the various egg quality parameters (both internal and external) to compare the effect of 0.033% (T2) and 0.066% (T3) BAG mixed layer feed against the feed with null treatment.

Egg production parameters

Table 1 and figure 1 demonstrates that the various of BAG levels had a substantial impact ($p<0.001$) on the feed conversion ratio (FCR), average egg weight (g), egg mass output (EMO) (g/b/d), and hen day egg production (%). In the case of HDEP (%), T2 was noticeably higher ($p<0.05$) than T3. For the trait, egg weight (g), T2 was significantly ($p<0.05$) better than both T1 and T2. Again, EMO (g/b/d) was higher ($p<0.05$) in T2 than in both T1 and T3, though T1 and T3 did not differ notably ($p>0.05$) with each other. In addition, FCR is similar ($p<0.05$) in T1 and T2, where both values were better than T3. During the study period, there was no mortality was recorded. So, the survivability for T1, T2, and T3 was 100%. Therefore, 0.033% performed comparably better in most of the laying parameters.

Dietary BAG supplementation boosted egg production and mass compared to non-supplemented chickens, which improved FCR values (Jahanian and Golshadi, 2015). Poultry fed with varying amounts of butyric acid gained more weight compared to those who didn't but feed intake, feed conversion ratio (FCR) and mortality did not differ significantly (Raza et al., 2017). Arabshahi et al. (2021) found that butyrate glycerides (BG) could intensify the effect of multi carbohydrase (MC) and led to improved egg-laying performance. The low pH environment in the gastrointestinal tract caused by BAG intake (Panda et al., 2009) may positively affect the activity of non-starch.

Table 1. Impact of varying butyric acid glycerides concentrations on the production performance of laying pullets (24 – 36 weeks)

Parameter	T_1	T_2	T_3	P-value	LS
HDEP (%)	87.93^b±0.83	93.38^a±0.68	92.14^a±0.73	0.018	**
Egg Weight (g)	58.04^c±0.34	60.85^a±0.26	59.81^b±0.37	0.017	**
EMO (g/b/d)	52.64^b±0.62	56.79^a±0.41	53.48^b±0.59	0.016	**
Feed Intake (g/b/d)	114.00±0	114.00±0	114.00±0	-	-
FCR	2.11^b±0.0	2.10^b±0.0	2.17^a±0.2	0.014	**
Survivability (%)	100	100	100	-	-

T1: Hand-made layer mash (control diet); T2: Control diet + 0.033% BAG; T3: Control diet + 0.066% BAG, n=number of days observed; means with different superscripts along the row differ significantly (P<0.05); **Significant at 0.1% level (p<0.01); LS= Level of significant.

polysaccharide degrading enzymes and improve nutrition utilization of laying hens. Butyric acid is also reported to improve the integrity of the epithelial cell line and the absorptive surface of the intestine by stimulation of cell proliferation and differentiation in the intestinal epithelium, leading to better nutrient utilization (Nari et al., 2020). Both egg production and egg mass were influenced by the interaction between the basal diet and BAG; in wheat-based diets, supplemental BAG was more beneficial (Jahanian and Rasouli, 2014a). Butyric acid does not considerably alter feed intake, according to several research. Adil et al. (2010) observed a noteworthy distinction in the body weight gain (BWG) and feed conversion ratio (FCR) between the birds given the organic acid diet and those fed the control diet. This difference suggests that the organic acid meal was more effectively absorbed and used. When researching butyric acid, inconsistencies often arise based on the kind of diet and the types of butyrate (calcium salt, sodium salt, glyceride, etc.). (Leonel and Alvarez-Leite, 2012; Kaczmarek et al., 2016). In an experiment with broilers, Kaczmarek et al. (2016) showed that butyric acid positively boosted BWG and FCR. Regardless of the age of the birds, the addition of 0.2 g/ kg and 0.3 g /kg of butyrate increased FCR. Additionally, we found that 0.33 gm/Kg outperformed the competitors in terms of results. On the other hand, 0.4 g kg-1 increased FCR and decreased feed intake (FI). When compared to the control and other butyrate doses, the investigation's results showed that the butyrate dose of 0.3 g kg-1 had the greatest beneficial benefits.

External egg quality parameters

Throughout the course of the experiment, the exterior quality of the egg parameters was evaluated four times. The findings are shown in Table 2. There was no discernible variation in any of the exterior egg quality features at 24-week, 28-week, 32-week, and 36-week intervals in the first, second, third, and fourth assessments of egg quality, owing to varying levels of BAG supplementations. Shape index and shell thickness did not demonstrate any significant dissimilation because to varying quantities of BAG in the diet, even though shell percentage increased significantly (p<0.001) at 28 weeks.

When sources of butyric acid were introduced to both the thickness and the percentage of eggshell to total egg weight of laying chickens who received their diet improved. Improved absorption of proteins and minerals could be the cause of this improvement. According to Soltan (2008), Rahman et al. (2008), and Grashorn et al. (2013a), adding organic acid resulted in an increase in eggshell thickness. However, in hens fed diets supplemented with a blend of organic acids,

Table 2. Effect of butyric acid glycerides on external egg quality parameters of laying pullets (24 – 36 weeks)

Age (Wks.)	Parameters	T_1	T_2	T_3	P-Value	LS
24	Shape Index (%)	75.91±0.97	78.21±1.35	77.23±0.91	0.37	NS
	Shell Percent (%)	9.97±0.30	10.59±0.43	10.56±0.13	0.33	NS
	Shell Thickness (mm)	0.35±0.02	0.38±0.01	0.39±0.01	0.14	NS
28	Shape Index (%)	76.65±2.37	77.39±1.48	72.02±1.76	0.15	NS
	Shell Percent (%)	9.17^{c}±0.57	10.10^{b}±0.20	11.11^{a}±0.16	0.01	*
	Shell Thickness (mm)	0.33±0.02	0.35±0.09	0.33±0.07	0.63	NS
32	Shape Index	75.91±1.00	78.21±1.35	77.23±0.91	0.37	NS
	Shell Percent (%)	9.97±0.30	10.59±0.43	10.56±0.13	0.33	NS
	Shell Thickness (mm)	0.35±0.020	0.38±0.01	0.39±0.01	0.14	NS
36	Shape Index (%)	73.93±0.93	77.57±1.48	74.52±2.05	0.26	NS
	Shell Percent (%)	10.05±0.29	10.46±0.36	9.73±0.40	0.38	NS
	Shell Thickness (mm)	0.38±0.01	0.39±0.07	0.40±0.08	0.32	NS

T1: Hand-made layer mash (control diet); T2: Control diet + 0.033% BAG); T3: Control diet + 0.066% BAG), n=number of days observed; means with different superscripts along the row differ significantly (P<0.05); *Significant at 0.5% level (p<0.05); LS = Level of significant; NS= non-Significant

Yesilbag and Colpan (2006) reported no increase in eggshell thickness. Kaya et al. (2014) found that eggshell strength increased with organic acid intake. Furthermore, research has demonstrated that lowering the food's pH may improve the eggshells' quality (Switkiewicz et al., 2010). Salts of butyric acid have differing impacts on the quantity and quality of eggs produced. Soltan (2008) and Sikandar et al. (2017) attribute this variance to the food's composition, the inclusion rate, the environment, and the source of butyric acid. Dietary supplementation with butyric acid could increase eggshell thickness in laying hens fed corn-soybean meal-based diets. This improvement could be explained by a greater increase in mineral and protein absorption after dietary supplementation with BAG (Arabshahi et al., 2021).

Internal measure of egg quality

The egg's interior quality was assessed based on the quality of the yolk and albumen. Table 3 displays the results of the internal quality of the eggs (AI, HU, YI, YCS, YP). The Haugh unit of eggs is correlated with albumen height, and and protein content of the egg are assumed to improve. All internal quality metrics did not exhibit statistically significant changes between laying hens that were 24 and 36 weeks old. However, when compared to the control, 0.033% BAG, and 0.066% BAG supplemented group, the Haugh unit (p<0.05) and albumen index (p<0.05) revealed significant changes at the 28th week of laying. Furthermore, at 32-week yolk percentages differ significantly among T1, T2, and T3.

In layer chickens fed an additional diet with 1% lactic acid (Gama et al., 2000), Yalcin et al. (2000) showed profound variations in albumen index and yolk index. In hens given 0.05% organic acid supplementation, they likewise observed a little decrease in haugh unit. However, according to Yesilbag and Colpan (2006), there was no discernible effect of dietary organic acid on the internal indices of egg quality. The Haugh unit, shape index, and yolk color score were unaffected by dietary organic acids (P>0.05). The decreasing albumen index can be explained by additional

Table 3. Effect of butyric acid glycerides on internal egg quality parameters of laying pullets (24 – 36 weeks)

Age (Wks.)	Parameter	T_1	T_2	T_3	P-Value	LS
24	Albumen Index	0.05±0.014	0.07±0.004	0.07±0.004	0.107	NS
	Haugh Unit	64.41±9.940	78.95±1.785	82.85±3.044	0.133	NS
	Yolk Index	0.41±0.020	0.42±0.021	0.41±0.008	0.954	NS
	Yolk Color Score	4.75±0.478	5.50±0.288	5.00±0.000	0.295	NS
	Yolk Percent (%)	23.38±1.422	22.86±1.055	23.92±1.452	0.854	NS
28	Albumen Index	0.08^{a}±0.010	0.06^{ab}±0.008	0.04^{b}±0.002	0.026	*
	Haugh Unit	79.96^{a}±4.102	76.49^{a}±4.462	59.40^{b}±4.004	0.015	*
	Yolk Index	0.36±0.007	0.38±0.012	0.37±0.006	0.439	NS
	Yolk Color Score	4.75±0.250	5.00±0.408	5.50±0.288	0.295	NS
	Yolk Percent (%)	26.09±0.527	23.49±1.279	23.73±0.857	0.151	NS
32	Albumen Index	0.05±0.014	0.07±0.004	0.07±0.004	0.107	NS
	Haugh Unit	64.41±9.940	78.95±1.78	82.85±3.044	0.133	NS
	Yolk Index	0.41±0.02	0.42±0.021	0.41±0.008	0.954	NS
	Yolk Color Score	4.75±0.478	5.50±0.288	5.00±0.00	0.295	NS
	Yolk Percent (%)	26.83^{a}±0.424	24.52^{b}±0.838	23.18^{b}±0.744	0.014	*
36	Albumen Index	0.06±0.006	0.08±0.010	0.06±0.005	0.383	NS
	Haugh Unit	77.35±3.384	80.26±5.505	74.99±4.00	0.706	NS
	Yolk Index	0.40±0.000	0.39±0.006	0.39±0.004	0.521	NS
	Yolk Color Score	5.50±0.288	6.00±0.408	5.50±0.288	0.499	NS
	Yolk Percent (%)	24.78±1.572	23.71±1.032	23.24±1.353	0.712	NS

T1: Hand-made layer mash (control diet); T2: Control diet + 0.033% BAG); T3: Control diet + 0.066% BAG), n=number of days observed; means with different superscripts along the row differ significantly ($P<0.05$); *Significant at 0.5% level ($p<0.05$); LS = Level of significant; NS= non-Significant

dietary organic acids ($P>0.05$). This outcome was reliable with that of Soltan (2008), who discovered that there was a little decrease in varying levels of organic acid incorporation in layer hens' albumen index. The hen's advanced age and the increased albumen percentage from supplementing with organic acids may be the source of the decreased albumen index. Wang et al. (2021) found that although *Clostridium butyricum* increased eggshell thickness and albumen height, butyric acid glycerides improved the Haugh unit and yolk color of eggs.

Conclusions

To conclude the dietary supplementation with butyrine glyceride indicates that BAG affect laying efficiency and egg quality. The BAG-treated groups showed better performance than the control diet group in entire laying period. When considered collectively, it is possible to draw a conclusion that incorporating 33g/kg BAG into a layer diet could improve the laying performance and their egg quality.

Acknowledgements
The authors would like to acknowledge ACI Animal Health, Dhaka, Bangladesh for the providing the butyrine glycerides (BAG) sample.

Authors contribution
Bapon Dey oversaw the whole research project, organized the study, and provided a critical evaluation of the report. Atikur Rahman Asif, Nusrat Jahan, Prantic Kumar Goswami, Sumaiya Koly, and Quazi Abir Hassan Roddur carried out the study and wrote the draft text.

Data availability
With the authors' permission, all relevant data used in this study will be made public.

Conflict of interest
The authors declare that there is no conflict of interest of any person, company or any aspect of the impact of the manuscript.

Consent to participate
The authors provide full consent to participate.

Consent for publication
All authors are fully agreed to publish this article in the Bangladesh Journal of Animal Science.

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