EFFECTS OF TRIBUTYRIN IN DIET ON EGG PERFORMANCE AND QUALITY OF HENS IN THE LATE STAGE OF LAYING CYCLE

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ABSTRACT

An experiment was conducted to determine the effect of diet supplementing with tributyrin (TB) in different levels in diet on egg performance and quality of laying hens from 55-65 weeks age. A total of 960 Hisex Brown laying hens at the 55 weeks of age were randomly distributed in a completely randomized design experiment, with 4 treatments and 3 replicates, each replicate consisted of a line with 20 pens (4 birds/pen). The experimental data was collected during 10 weeks. Treatments used: (1) Control (Cont): Basal diet (B) without any supplementation; (2) TB0.5: B + Tributyrin at 0.5 g/kg feed; (3) TB0.75: B + Tributyrin at 0.75 g/kg feed; (4) TB1.0: B + Tributyrin at 1.0 g/kg feed. The results showed that, average daily feed intake was not affected by tributyrin supplementations in the diets (P>0.05). But a little improvement hen day production, egg mass and egg weight in TB0.75 and TB1.0 compared to TB0.5 and Cont group. Tributyrin supplementation significantly increased eggshell thickness (P < 0.01), and the higher yellow color of egg york was found in chicken eggs fed TB0.75 and TB1.0 treatments. In conclusion, adding tributyrin at 0.75 and 1.0g/kg feed could trend lightly improved hen day production, egg shell thickness, and egg york color and feed conversion ratio compared to control group of laying hen in the late state of laying cycle.

Keywords: *Hen- day production, Hisex Brown, laying hen, Tributyrin*

INTRODUCTION

Commercial laying hens are popular raising in many laying farms in the Mekong Delta of Viet Nam. In the present, antibiotics are not allowed to include in the animal feed, it lead to increase the proportion of sick and dead chickens in many laying farms. In addition, Hisex Brown is a popular breed, which have earlier age at first lay (19-21 weeks age), then slowly reach the peak at around 28 to 32 weeks age, this laying hens are normally culled after 72-76 weeks of age due to low egg production (Haider and Nath, 2014). Therefore, in order to reduce the physiological reducing of egg production in the late stage of hen laying cycle, there are not only considering the quantity of feed but also the supplement factors. Thus, the study found the solution for replace antibiotics in the laying hen diets are really becoming an urgent need. The feed additive producers have researched and supplied many kinds of replacement products, in which organic acids are researching. Organic acid may enhance the digestive microflora with beneficial microorganisms, inhibits the desease cause of harmful bacteria in poultry (Sakdee et al., 2018). Research done by Nguyen Thi Thuy and Nguyen Cong Ha (2018) found that supplementation of mixture of organic acid at 0.1 g/kg feed could lightly improve hen day production and feed conversion ratio, but without egg quality of commercial laying hens. Tributyrin is a product that supply acid butyric, there are few studies on using butyric acid in the form of tributyrin in laying hens up to now. Therefore, this study was conducted to evaluate the effect of tributyrin in different levels in diet on egg production, egg quality parameters during the late stage of laying hens.

MATERIALS AND METHODS

Materials

Animals and experimental design

The experiment was conducted in a laying farm in O Mon district, Can Tho city from July to October, 2020. The hen house was a tunnel ventilated house with 3 floor cages in a line

inside. A total of 960 Hisex Brown laying hens were housed in cages (pen). The experimental time lasted 10 weeks, from 55 to 65 week old of hens. During the experimental period, hens were supplied water *ad-libitum*. Feed formulation and composition are showed in Table 1, and hens were fed twice a day. Tributyrin product, which have 50% of acid butyric was a white powder, and provided by Menon Animal Nutrition Technology Co., Ltd, with the main ingredients of butyric acid. The study was arranged as a completely randomized design with 4 treatments and 3 replicates, each replicate consisted of a line with 20 pens (4 birds/pen).

Experimental treatments and feed

Table 1. Feed formulation and composition of the basal diet

Ingredients, %	Basal diet
Rice bran	14.0
Maize	47.0
Broken rice	10.0
Soya bean meal	18.0
Fish meal	7.0
Premix-vitamin[1]	1.0
Limestone	3.0
Chemical composition, %	
DM	88.4
Crude protein	16.5
Ether extract	5.15
Ash	10.4
Crude fiber	5.10
Ca	3.11
P	1.05
NaCl	0.2
NFE	63.0
ME (MJ/kg feed)	11.5

The basal dietary was formulated following laying hen requirement with amount of metabolizable energy (11.5 MJ/kg) and crude protein (16.5 %) content. Feed ingredients in basal diet included: Maize, broken rice, rice bran, fish meal, soya bean meal, amino acids and premix vitamin. The supplementation products were supplied in the diets every day.

Treatments were:

Cont: Basal diet (B) without any supplemented product in the diet

TB0.5: B + Tributyrin 0.5g/kg feed

TB0.75: B + Tributyrin 0.75g/kg feed

TB1.0: B + Tributyrin 1.0g/kg feed

Measurement Methods

Egg performance

Hen day production, averages daily feed intake, and egg weight were recorded daily for computing the average daily egg production, egg mass and feed conversion ratio. Egg mass was determined by calculating hen day production x egg weight. Feed conversion ratio was

determined by calculating feed intake (g)/egg mass (g). Egg classification (broken, unnormal, double york egg) was separated for computing each class percent from the total egg production.

Egg quality

At the 65 weeks old, 60 eggs/treatment (1 egg/pen) were randomly collected for egg quality analysis. Egg shape index was determined by calculating (egg width / egg length) x 100, the same eggs were broken to weight albumin, york and shell individually (Englmaierová et al., 2014). Shell thickness was determined by calculating the mean of triplicate measurement from different sides of shell (Güçlü et al., 2008). Haugh Unit was measured using formula HU=100 x log (H - 1.7 $W^{0.37}$ + 7.57), in which H is albumen height (mm) and W is egg weight (g) (Saleh, 2013). York color was recorded using a colorimeter, which indicated degrees of lightness of a york sample (L), red-ness (a) and yellow-ness (b).

Statistical analysis

Data was analyzed by ANOVA using the General Liner Model (GLM) of Minitab Statistical Software Version 16. Tukey pair-wise comparisons were used to determine differences between treatment means at $P<0.05$.

The statistical model used is as follows: $Y_{ij} = \mu + \alpha_i + e_{ij}$

Where Y_{ij} is egg performances or egg quality; μ is overall mean averaged over all treatments; α_i is effect of treatment; e_{ij} is random error associated with treatment and replicate within treatments.

The chemical composition of basal feed was determined following Association of Official Analytical Chemists methods (AOAC, 1990). York color was recorded using a colorimeter (Chromameter Minolta, CR-400 Head, DP-400/ Japan), which indicated degrees of lightness of a york sample (L), red-ness (a) and yellow-ness (b).

RESULTS AND DISCUSSIONS

Egg performance and feed efficiency

Egg production, feed intake and feed conversion ratio of laying hens during the period between 55 and 65 weeks of age are shown in Table 2. Results showed that the supplement of tributyrin at 0.75 and 1.0 g/kg feed trend to lightly increase egg production but not with daily feed intake of hens, lead to better feed conversion ratio of egg to compare with control and TB0.5 treatments, but all the differences were not significant. However, this may also explain that tributyrin in the term of acid butyric could reduce intestinal pH, increased enzyme activity, so it should improve the digestion and absorption of protein, then improve egg production (Sakdee et al., 2018).

In this research, feed intake of hens in TB0.5 treatment was little lower than TB1.0 treatment around 2 g/day, this is quite small number. However, feed conversion ratio in all supplemented treatments were very little higher than that of control treatment. It may be because supplementation of an organic acid may increase intestinal villi height in all segments of the small intestine, especially in the ileum, thereby improving the absorption and increased feed efficiency (Sheikh et al., 2011). Moreover, tributyrin is a triglyceride which not only supplies energy, but also provides butyric acid for the development and maintenance of the epithelium of the intestinal tract (Sakdee et al., 2018), and tributyrin contain three molecules

of butyrate that can be broken down by lipase and then release a large amount of butyrate into the intestine (Miyoshi et al., 2011), and can bypass the stomach to reach the hind gut (Augustin et al., 2011), so feed efficiency of tributyrin supplementation have trend better than control.

Table 2. Average egg production and feed intake of laying hens during 55-65 weeks age

Variables	Treatments				SEM	P
	Cont	*TB0.5*	*TB0.75*	*TB1.0*		
Feed intake, g/bird/day	126.1	125.5	126.2	127.2	4.424	0.26
Egg production, %	75.1	75.8	76.7	76.6	1.214	0.21
Egg weight, g/egg	61.1	60.6	61.5	61.7	0.223	0.24
Egg mass, g/bird	45.8	45.9	47.2	47.3	0.605	0.08
Feed conversion ratio, g feed/g egg	2.75	2.73	2.67	2.69	0.068	0.27
Cost (VND)/kg egg	27,500	27,436	26,900	27,169	-	-

Cont: Basal feed (B)+ No supplementation; TB0.5: B+ Tributyrin 0.5g/kg feed; TB0.75: B+ Tributyrin 0.75g/kg feed; TB1.0: B + Tributyrin 1.0g/kg feed; Basal feed price: 10,000 VND/kg; Tributyrin:100,000 VND/kg

The results in Table 2 also shows that egg weight and egg mass have trend little higher in TB0.75 and TB1.0 diets to compare with TB0.5 treatment, this is in agreement with research from Sakdee et al. (2018), who found the egg weight increased in higher organic acids supplementation diet. This result also was consisted with research by Rahman et al. (2008), who found that the feed conversion ratio improved in the laying hens when their diet was supplemented with organic acids. And research from Nguyen Thi Thuy et al. (2018) showed similar results when supply with mixture of organic acids showed higher egg production and weight.

Table 3. Egg production of laying hens from 55-65 weeks age

Weeks of age	Treatments				SEM	P
	Cont	*TB0.5*	*TB0.75*	*TB1.0*		
56	81.7	83.2	82.1	83.1	2.142	0.12
57	80.0	81.0	82.0	82.0	2.111	0.11
58	80.1	81.0	82.1	81.0	1.877	0.28
59	76.4	77.3	78.3	78.2	1.976	0.44
60	75.2	74.4	77.4	76.0	2.022	0.26
61	74.5	74.5	75.0	75.0	1.677	0.76
62	72.1	74.5	74.0	74.0	2.111	0.08
63	71	72.0	72.3	72.2	3.001	0.25
64	70.2	70.2	72.1	71.1	2.112	0.17
65	70.0	70.3	72.1	73.1	1.978	0.23

Cont: Basal feed (B)+ No supplementation; TB0.5: B+ Tributyrin 0.5g/kg feed; TB0.75: B+ Tributyrin 0.75g/kg feed; TB1.0: B+ Tributyrin 1.0g/kg feed

Weekly egg production of laying hens during the period between 55 and 65 weeks of age is presented in Table 3. The egg production of the Hisex Brown laying hens was slowly reducing with the increasing of hen age from 55 to 65 weeks age. This is in agreement with the report of Seidler (2003) who showed that, egg production of commercial laying hens often

start to slowly decrease around over 40 weeks of age. The reducing of egg production is quick or slow dependence on nutrition and management that laying hens received. These results demonstrated that because of tributyrin supplementation in the diet made the slowly reducing of egg production after 50 weeks of age.

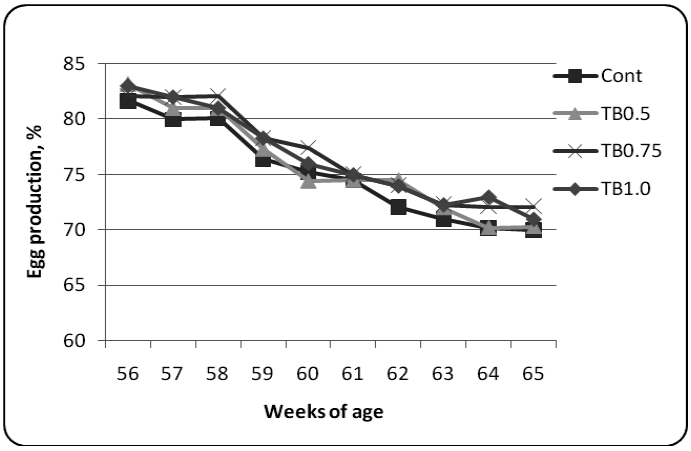

Figure1. Egg production of laying hens from 56-65 weeks of age

Egg classification

Table 4. Effect of tributyrin supplementation on broken eggs, unnormal eggs and average live gain of laying hens

Variables	Treatments				SEM	P
	Cont	*TB0.5*	*TB0.75*	*TB1.0*		
Broken egg proportion,%	3.52[a]	1.85[b]	1.65[b]	1.82[b]	0.301	0.03
Unnormal egg, %	0.39	0.38	0.30	0.22	0.101	0.26
Double york egg,%	1.35[c]	2.41[b]	3.22[a]	3.12[a]	0.328	0.04
Hen average live gain, g/day	5.21	5.97	5.10	5.22	0.610	0.17

Cont: Basal feed (B)+ No supplementation; TB0.5: B+ Tributyrin 0.5g/kg feed; TB0.75: B+ Tributyrin 0.75g/kg feed; TB1.0: B+ Tributyrin 1.0g/kg feed

Effect of tributyrin supplements on broken egg, unnormal egg and double york egg proportion of laying hens during the period between 55-65 weeks age are presented in Table 4. The proportion of broken eggs was lower in tributyrin supplementation diets than control diet, in contrast with double york eggs. It means that the highest double york eggs were for hens fed TB0.75 and TB1.0. So, when tributyrin was added in the diets tended to improve the proportion of double york eggs.

Egg quality

Egg quality of laying hens are presented in Table 5, the egg quality parameters are important factor in the economic profitability of egg production. Normally, the internal egg quality and eggshell quality decline with the increasing age of hen, due to the decrease in nutrient absorption for eggshell formation and the increased in egg size and shell surface area (Roberts et al., 2013). The egg shape, york and albumin index did not differ from eggs of hens in different treatments, but a little increased egg shell thickness of hens supplied with higher levels of tributyrin supplementation. This is similar with results from Hatice et al. (2014), who reported that supplementation of organic acid can causes higher eggshell thickness. The

results of this experiment was inconsistent with the study of Soltan (2008), who found that the eggshell thickness was improved in laying hens fed organic acids 0.078% supplemented compared with control treatment.

Table 5. Egg quality of laying hens supplemented with tributyrin from 55-65 weeks age

Variables	Treatments				SEM	P
	Cont	*TB0.5*	*TB0.75*	*TB1.0*		
Egg weight, g	60.6	61.1	60.7	61.0	0.558	0.25
Egg shape index	78.2	77.1	78.5	78.4	0.311	0.51
Egg shell thickness, mm	0.37^{b}	0.38^{ab}	0.39^{a}	0.39^{a}	0.030	0.04
York index	0.46	0.47	0.46	0.47	0.004	0.43
Albumen index	0.08	0.09	0.08	0.08	0.007	0.06
Haugh unit (HU)	82.5	81.8	81.9	81.6	0.758	0.07
Yolk color						
L*	48.4	47.6	48.3	48.2	0.521	0.34
a*	6.62	6.81	6.22	6.31	0.264	0.23
b*	43.2^{b}	43.4^{b}	44.1^{b}	45.1^{a}	0.218	0.03

Cont: Basal feed (B)+ No supplementation; TB0.5: B+ Tributyrin 0.5g/kg feed; TB0.75: B+ Tributyrin 0.75g/kg feed; TB1.0: B+ Tributyrin 1.0g/kg feed

Hence, tributyrin may be valuable and useful for egg producers in terms of the improvement in egg shell quality and gut health. It may be because butyric acid in tributyrin is quickly absorbed and metabolized by mucosa cells; the absorption and metabolism of butyric acid begins in the mucosa of the crop and this process continues throughout the gastro intestinal tract, and also is active in the small intestine and encapsulated butyrate is active the ceca and colon (Moquet et al., 2017). This result also similar with report of Kaya et al. (2015) who have been reported the supplementation of organic acids or short chain fatty acids positively improved the eggshell quality, and tributyrin supplementation significantly increased the eggshell thickness and tended to increase the eggshell percentage (Dani-el Ruth Hanna, 2019). However, yolk color was higher in TB1.0; TB0.75 and TB0.5 to compare with Cont hens. It may be because of tributyrin would increase the absorption of xanthophyll concentration in feed (Soltan, 2008).

CONCLUSIONS

Supplementation of tributyrin at levels at 0.75 and 1.0g/kg feed could lightly improve hen day production, egg shell thickness, egg york color and feed conversion ratio compared to 0.5 g/kg feed and control group of Hisex Brown laying hen in the late state of laying cycle.

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