Original Article

Effect of adding tributyrin in diet on egg production, egg quality, and gastrointestinal tract in laying hens after peak period

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Abstract

This study was conducted to evaluate the effects of supplementing diet with tributyrin on productive performance, egg quality and gastrointestinal tract of laying hens during the post-peak period of egg production. In total, 384 Lohmann Brown-Classic hens aged 56 weeks were used for the 16-week experiment. The hens were divided into two groups, each group consisting of 8 replications of 24 hens each. Experimental diets were supplemented with tributyrin at the level of 0.00% (control diet) and 0.05%. Tributyrin supplementation significantly increased eggshell thickness ($P<0.01$) and tended to increase eggshell weight ($P=0.07$). However, it had no significant effect on production performance, pH in each part of the gastrointestinal tract, small intestinal morphology, populations of *E. coli* and *Lactobacillus* spp. and short chain fatty acids concentrated in the caecum of laying hens. It was concluded that tributyrin supplementation in the diet improved the eggshell thickness after the peak rate of lay, although significant effects on egg production and the gastrointestinal tract were not observed.

Keywords: egg production, eggshell, gut conditions, laying hens, tributyrin

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Introduction

It is generally known that egg production and eggshell quality of laying hens decrease as the hens age (Al-Batshan et al., 1994; Soltan et al., 2008; Swiatkiewicz et al., 2010; Kocevski et al., 2011; Menezes et al., 2012; Roberts et al., 2013). These outcomes are probably due to the impaired quality of the mucosal cells in the intestine, the decreased length of the intestinal villi and the decreased absorption of nutrients (Rahman et al., 2008). Particularly after the peak period of laying eggs, the internal egg quality and external (eggshell) quality generally decline (Al-Batshan et al., 1994; Kocevski et al., 2011; Menezes et al., 2012; Roberts et al., 2013) due to the increase in egg weight without an increase in the amount of calcium carbonate deposited in the shell (Nys, 2001) or/and some dysfunction of the shell gland (Joyner et al., 1987). Therefore, from the post-peak period to the end of egg production, the incidence of cracked eggs has been reported as 6-20% (Washburn et al., 1982; Roland and David, 1988; Nys, 2001).

In old layers and broiler breeder hens, several investigators reported that supplementing diet with organic acids improved the laying performance and eggshell quality (Yesilbag and Colpan, 2006; Sengor et al., 2007; Soltan, 2008). Since butyric acid is considered as an acidifier and energy source in the gastrointestinal tract, it serves as a primary source of energy for colonocytes, a strong mitosis promoter and a differentiation agent in the gastrointestinal tract (Salminen et al., 1998). Under good hygiene conditions, Mahdavi and Torki (2009) and Adil et al. (2010) reported that supplementing diet with butyric acid increased the serum Ca in broiler chickens, although the productive performance was not improved. Therefore, giving butyric acid to old layer hens may improve eggshell quality. However, there are some difficulties with the addition of butyric acid in feed such as its high volatility and unpleasant odor (Antongiovanni et al., 2010). The odor is a result of the triglyceride of butyric acid (tributyrin) being produced (Antongiovanni et al., 2010). Butyrate can be released from tributyrin by intestinal lipase and then is absorbed by the small intestine (Li et al., 2015). Hence, tributyrin may be valuable and useful for egg producers in terms of the improvement in egg shell quality and gut health.

Furthermore, the use of antibiotics in animal feed is restricted due to concerns of antibiotic residues in animal products. In the European Union (EU), antibiotics have been banned as a growth promoter in animal feed since 2006. Consequently, the development of alternative feed additives to replace the use of such antibiotic growth promoters has been a high priority (Gharib Naseriet al., 2012; Saki et al., 2012; Youssef et al., 2013; Kaya et al., 2015; Lee et al., 2015). In particular, the potential of organic and short chain fatty acids (including butyric acid) as antimicrobials has been reviewed in detail by Ricke (2003). Moreover, there is evidence that dietary supplementation of 0.05% tributyrin inhibited the proinflammatory cytokines release, improved the intestinal energy status, and enhanced the intestinal anti-oxidative capacity in LPS-challenged broilers (Li et al., 2015). Although there is evidence that tributyrin can improve the trophic status of the epithelial mucosa in the gut of nursery pigs (Piva et al., 2002) and the production performance or carcass trait of broiler chickens (Antongiovanni et al., 2010), there have been few reports on the effect on the bacterial population in laying hens.

The supplementation of tributyrin as a source of butyric acid may improve gut physiology and ecology, and consequently promote egg production and eggshell quality of old laying hens. According to Li et al. (2015) who reported the positive effects of supplementing tributyrin at 0.05% of diet on immune response of broiler, and commercial recommendation level of tributyrin (0.025 to 0.10% in diet), hence this study was conducted to evaluate the effect of supplementing diet with tributyrin (0.05%) on egg production, eggshell quality, gastrointestinal pH, large intestine selected microflora, intestinal morphology and short-chain fatty acid concentration in the ceacum of laying hens in their post-peak production period (50-65 weeks of age).

Materials and Methods

Animals and Management: This study was conducted in line with the animal ethics guidelines of the Research Policy of Kasetsart University, Thailand. In total, 384 Lohmann Brown-Classic hens aged 50 weeks were used for the 16-week experiment. Under thermoneutral conditions, the hens were housed in wire cages with four hens each and six adjacent cages were used as one replication. The hens were divided into 16 units and each unit consisted of 24 hens. All 16 units were randomly divided into two groups, each group consisting of eight replications. A t-test was used to compare measurement values obtained from the two independent groups.

During the 16-week experimental period, the post-peak egg production of the laying hens (from 50 to 65 weeks of age) was kept, maintained and treated in adherence with the accepted standard for the humane treatment of animals under an evaporative cooling system to control air ventilation and temperature. Thus, the average housing temperature throughout the experimental period was 25.88°C. The lighting program was 16 hours from 5 am to 9 pm daily. Feed was offered twice daily (8 am and 4 pm) *ad libitum* and water was provided *ad libitum* from water nipples.

Experimental diets: Nutrient composition of the experimental diets was determined according to the recommendations for the Lohmann Brown-Classic breed. Two experimental diets (mash form) were formulated with different levels of tributyrin, 0.00% (the control) and 0.05% (Table 1), which were prepared using a commercial feed mill in mash form. The level of tributyrin powder in the diet (0.05%) of laying hens in this study was supplemented according to the commercial dosage advice (0.025-0.10% in diet) and Li et al. (2015).

Table 1 Ingredient and calculated chemical composition of basal diet (as fed basis)

Item	Control (Tributyrin 0.0%)	Tributyrin 0.05%
Ingredient (%)		
Corn	62.405	62.405
Rice bran oil	1.449	1.449
Soybean 48% CP	23.310	23.310
DL-Methionine	0.103	0.103
Mono-Dicalciumphosphate 21%P	1.535	1.535
Calcium carbonate	9.647	9.647
Salt	0.054	0.054
Vitamin & Mineral premix[1]	0.500	0.500
Choline Chloride 60%	0.077	0.077
Tributyrin	-	0.050
Sodium bicarbonate	0.870	0.870
Inert filler (corn starch)	0.050	-
Calculated chemical composition		
ME. for Poultry (Cal/Kg.)	2,750.000	2,750.000
Protein (%)	16.320	16.320
Fat (%)	3.914	3.914
Calcium (%)	4.000	4.000
Available Phosphorus (%)	0.370	0.370
Lysine, Digestible (%)	0.730	0.730
Methionine + Cystine, Digestible (%)	0.570	0.570

[1]Premix: Lutavit Mix CNK004 consists of Vitamin A 4.80 MIU, D_3 0.96 MIU, E 3.20 g, K_3 0.80 g, B 0.40 g, B_2 1.60 g, B_6 1.20 g, B_{12} 0.004 g, Pantothenic acid 3.80 g, Niacin 6 g, Folic acid 0.20 g, Biotin 0.036 g, Se 0.04 g, Fe 24 g, Mn 24 g, Zn 16 g, Cu 2.40 g, I 0.14 g, Feed preservative substance 2.50 g, Flavor 10 g and carrier added to 1.00 kg premix.

Measurement

Production Performances: Throughout the experiment, the hen-day egg production and egg weight were recorded daily. The percentage of egg production, egg weight, feed intake, egg mass (g/hen/day), and feed conversion ratio (FCR; kg feed/kg egg mass) were determined at four-week intervals. The feed intake was calculated per replication and period on the basis of the total feed provided minus the total feed leftover. The egg mass was calculated by multiplying the egg weight by the hen-day egg production percentage. The feed conversion ratio was calculated as grams of feed per day per hen divided by the gram egg mass per day per hen.

Eggshell Quality and Internal Egg Characteristic: At 4-week intervals, all eggs from each replication were weighed and four eggs from each replication having a weight close to the replication's mean were chosen to analyze eggshell qualities and characteristics consisting of eggshell breaking strength (using an Eggshell Force Gauge Model II, Robotmation Co., Ltd., Tokyo, Japan), eggshell thickness (using an electronic digital micrometer) and percentage of eggshell (relative eggshell weight calculated from eggshell weight divided by egg weight and multiply by 100).

Internal egg quality was measured as the percentage of albumen, percentage of yolk, albumen height (using a tripod micrometer) and yolk color (using a Roche yolk color fan). Egg freshness was indicated in Haugh units (HU) and calculated using $HU = 100\log[H - 1.7W^{0.37} + 7.57]$ (Eisen et al., 1962), where W refers to the egg weight (g) and H refers to the albumen height (mm).

pH in Gastrointestinal Tract and Caecum Bacterial Count: At the end of the trial, one hen of each replication was put down using CO_2 asphyxiation in an atmosphere of less than 2% oxygen (air displaced by CO_2). Immediately, the pH of contents in each part of the gastrointestinal tract (stomach, duodenum, jejunum, ileum, caecum, colon and rectum) was directly measured using a pH meter (IQ Scientific Instruments, Carlsbad, CA, USA).

The contents in the caecum were collected immediately from one hen of each replication after exsanguination, placed into sterile centrifuge tubes, put on ice and transported (within one hour after collection) to the laboratory for bacterial enumeration.

DNA Extraction: DNA extraction was carried out according to the studies of Chen and Kou (1993), Yu and Morrison (2004), and Chanyalew and Loongyai (2013). A sample of 0.05 g of caecal digesta was transferred into a 2 mL Eppendorf tube. Then, the sample was washed with 0.9% NaCl and cell lysis was achieved by beading in the presence of 300 µL of solution I [0.03 M Tris-HCl, 0.2 M Sucrose, 0.1 M NaCl and 0.01 M EDTA (pH 8.0)] and 600 µL of solution II [50mM Tris-HCl, 50 mM EDTA and 2.5% SDS]. Then, 300 µL of solution III [5M potassium acetate, glacial acetic] and solution IV [CH_2Cl_2:IAA in the ratio of 24:1] were added into the tube, followed consecutively by gentle hand shaking, incubation at -20°C for 15 min and centrifugation at 14,000 rpm and 4°C for 15 min. The supernatant was carefully transferred into a new

1.5 mL Eppendorf tube, 400 µL isopropanol was added and mixed well, followed by incubation at -20°C for 30 minutes, centrifugation at 14,000 rpm and 4°C for 10 minutes, then the supernatant was removed. The sample was washed with 500 µL of 70% ethanol, centrifuged at 14,000 rpm and 4°C for 5 min and the supernatant removed and dried at room temperature for approximately 1 hr. Finally, the DNA obtained was dissolved in 10-20 µL of distilled water.

Primers and Real-Time Polymerase Chain Reaction (Real-Time PCR): The targeted populations were *E. coli* and *Lactobacillus*. All primer set sequences are shown in Table 2. The total volume (10 µL) of PCR reaction mixture contained 1 µL of DNA template, 0.5 µL of each primer, 6 µL of deionized water and 2 µL of 5x HOT FIREPol® qPCR Mix Plus (no ROX). HOT FIREPol® qPCR Mix Plus (no ROX) comprised all the components necessary to perform qPCR including the HOT FIREPol® DNA polymerase, ultrapure dNTPs, $MgCl_2$ and EvaGreen® dye. The real-time PCR conditions for the *E. coli* were: denaturation for 15 min at 95°C followed by 40 cycles of denaturation at 95°C for 30 s, annealing at 60°C for 30 s and extension at 72°C for 30 s, with a final 10 min incubation at 72°C for the completion of primer extension after the last cycle. For the *Lactobacillus* samples, the real-time PCR conditions were similar to those for the *E. coli* except for the annealing temperature which was 58°C. Absolute quantification was achieved using Ct values, standard curves were constructed by amplification of known amounts of target DNA, and results were expressed as log_{10} copies per gram of sample.

Table 2 PCR primer sets for real-time PCR.

Target species	GenBank accession number	Primer sequence (5′-3′)	Size (bp)	References
E. coli	CP024278.1	F: GCG AAA ACT GTG GAA TTG GG R: TGA TGC TCC ATA ACT TCC CTG	252	Cebula et al., 1995
Lactobacillus spp.	CP026097.1	F: AGC AGT AGG GAA TCT TCC A R: CAC CGC TAC ACA TGG AG	341	Ponnusamy et al., 2011

Morphology of Small Intestine: Tissue samples were collected from the duodenum, jejunum and ileum and were immediately fixed in 10% neutral buffered formalin. Then, the tissues were carefully embedded in paraffin. For each specimen, at least 10 sections of 5 µm thickness were prepared. The tissues were then stained with haematoxylin-eosin for histological evaluation**.** Histology of the duodenum, jejunum and ileum tissue was assessed under a light microscope in accordance with Nunez et al. (1996).

Morphology of the small intestine in this study was described using the villus height, the crypt depth and the villus height to crypt depth ratio, which were determined using a computer-assisted image-analysis system (Biowizard, Thaitec, Thailand). The villus height was measured from the villus tip to the crypt-villus junction. The crypt depth was measured from the crypt-villus junction to the basolateral membrane and was recorded as the mean of 10 fields for each specimen.

Concentration of Short-Chain Fatty Acids (SCFAs) in Caecum: Contents in the caecum were immediately collected after the hens were exsanguinated. The samples were prepared by the modified method of Meimandipour et al. (2010) and Krutthai et al. (2015). A 1.5 ml aliquot of each sample was resuspended in 3 ml of sterile milli-Q water and then centrifuged (model MX-301; TOMY Kogyo, Tokyo, Japan) in microcentrifuge tubes at 14,000×*g* and 4°C for 10 minutes. Then, 900 µL supernatant of each sample was acidified using 100 µL 50 mM H_2SO_4, mixed by vortex for 30 seconds and then left to stand at room temperature. The samples were centrifuged at 14,000×*g* at 4°C for 10 minutes and the supernatant was analyzed for SCFAs using high performance liquid chromatography (HPLC).

The HPLC system consisted of an Agilent 1100 series (Agilent Technologies), Photodiode Array Detector (Agilent Technologies), Razex ROA-Organic Acids H+ (8%) Column (300 × 7.8 mm) and Razex ROA-Organic Acids H+ (8%) Guard Column (50 × 7.8 mm). The supernatants were filtered with a 0.22 µm nylon syringe filter (13 mm diameter; No. 2166) (Alltech Associated Inc., Deerfield, IL, USA) before injection into HPLC according to the method of Knarreborg et al. (2002). An amount of 20 µL of each sample was injected into the HPLC with autosampling and 0.005 N H_2SO_4 as the mobile phase. The running conditions provided for a column heat of 60°C, a flow rate of 0.5 ml/minute and the absorbance detector was operated at a wavelength of 210 nm. A mixture of succinic acid, lactic acid, formic acid, acetic acid, propionic acid, butyric acid and valeric acid was included as a standard in all analyses. Qualitative acid analysis was determined by the retention time of acid peaks, while quantitative analysis was carried out using a standard curve composed of the various acid concentrations.

Statistical Analysis: A t-test was used to compare measurement values obtained from the two independent groups on productive performance, egg quality, gastrointestinal pH, populations of *E. coli* and *Lactobacillus* spp., intestinal morphology and concentration of short-chain fatty acids in the caecum of laying hens using the Statistical Analysis System (SAS) Version 9.0, 2002 software package. The microbial populations were log transformed before statistical analysis. Statements of statistical significance were based on $P<0.05$.

Results

The effects of tributyrin supplementation on the productive performance and egg quality of laying

hens are presented in Table 3. The result reveals that the tributyrin supplementation in diet had no significant effects on the average egg production, egg weight, egg mass, feed intake and FCR of the laying hens throughout the experimental period. However, the tributyrin supplementation significantly increased the eggshell thickness (P<0.01) and tended to increase the eggshell weight (P=0.07), while yolk color, albumen weight, yolk weight, albumen-yolk ratio and Haugh units of the laying hens were not affected.

The effects of tributyrin supplementation in the diet on the gastrointestinal pH and bacteria in the caecum of laying hens are shown in Table 4. There were no significant effects of tributyrin on the pH in each part of the gastrointestinal tract of the laying hens, nor in the populations of *E. coli* and *Lactobacillus* spp. in the caecum of the laying hens.

The effects of tributyrin supplementation in the diet on the intestinal morphology of laying hens are shown in Table 5. There were no significant effects of tributyrin on the villus height and crypt depth of the duodenum, jejunum and ileum, nor in the ratio of the villus height to crypt depth in each segment of the small intestine .

The effects of tributyrin supplementation in the diet on the concentration of short-chain fatty acids in the caecum of laying hens are shown in Table 6. There were no significant effects of tributyrin on the concentration of succinic acid, lactic acid, formic acid, acetic acid, propionic acid, butyric acid and valeric acid in the caecal digesta of the laying hens.

Table 2 PCR primer sets for real-time PCR.

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Lactobacillus spp.	CP026097.1	F: AGC AGT AGG GAA TCT TCC A R: CAC CGC TAC ACA TGG AG	341	Ponnusamy et al., 2011

Table 3 Effects of dietary tributyrin supplementation on production performance and egg quality in 50- to 65-week-old laying hens.

Item	Control	Tributyrin 0.05%	P-value
Hen-day egg production (%)	93.22 ± 1.60	92.70 ± 2.56	0.64
Egg weight (g)	67.36 ± 0.62	67.26 ± 0.97	0.81
Egg mass (g)	62.78 ± 0.82	62.34 ± 1.66	0.51
Average daily feed intake (g/hen/day)	122.51 ± 1.62	121.89 ± 2.60	0.58
Feed conversion ratio (FCR) (kg feed/kg egg mass)	1.95 ± 0.03	1.96 ± 0.07	0.92
Shell breaking strength (N)	35.90 ± 2.80	35.97 ± 1.55	0.95
Albumen height	8.64 ± 0.34	8.59 ± 0.36	0.77
Yolk color	8.53 ± 0.15	8.66 ± 0.13	0.10
Shell thickness (μm)	328.81 ± 6.49	339.00 ± 6.15**	0.01
Albumen weight (%)	65.22 ± 0.65	64.98 ± 0.67	0.48
Yolk weight (%)	25.21 ± 0.60	25.31 ± 0.64	0.76
Eggshell weight (%)	9.57 ± 0.13	9.71 ± 0.17	0.07
Albumen-Yolk ratio	2.59 ± 0.09	2.58 ± 0.10	0.73
Haugh unit	90.86 ± 1.87	90.70 ± 2.03	0.87

**Significantly different (P<0.01). Values are presented as mean ± SD.

Table 4 Effects of tributyrin supplementation on pH of gastrointestinal tract and bacteria in caecum of 65-week-old laying hens

Item	Control	Tributyrin 0.05%	P-value
Gastrointestinal pH			
Crop	4.76 ± 0.05	4.76 ± 0.05	0.97
Proventiculus	4.50 ± 0.17	4.28 ± 0.17	0.39
Gizzard	3.88 ± 0.21	3.93 ± 0.21	0.85
Duodenum	5.90 ± 0.04	5.84 ± 0.04	0.24
Jejunum	5.76 ± 0.04	5.72 ± 0.04	0.42
Ileum	6.24 ± 0.24	6.30 ± 0.24	0.85
Caecum	6.18 ± 0.15	6.27 ± 0.15	0.68
Colon	6.43 ± 0.16	6.18 ± 0.18	0.32
Bacteria Count in Caecum			
E. coli (log_{10}CFU/g)	9.82 ±1.14	9.45 ± 0.59	0.43
Lactobacillus spp. (log_{10}CFU/g)	12.56 ± 3.27	12.79 ± 3.48	0.62

Values are presented as mean ± SE.

Table 5 Effect of tributyrin in diet on intestinal morphology of 65-week-old laying hens

Item	Control (0.0%)	Tributyrin 0.05%	*P*-value
Villous height			
Duodenum (μM)	891.74 ± 29.26	863.28 ± 32.05	0.53
Jejunum (μM)	765.76 ± 49.18	773.87 ± 49.18	0.91
Ileum (μM)	395.51 ± 35.99	444.29 ± 27.21	0.31
Crypt depth			
Duodenum (μM)	262.60 ± 24.02	298.44 ± 24.02	0.32
Jejunum (μM)	218.37 ± 18.36	229.10 ± 18.36	0.69
Ileum (μM)	173.17 ± 12.60	172.00 ± 9.52	0.94
Villous height:Crypt depth			
Duodenum (μM)	3.41 ± 0.20	2.95 ± 0.20	0.15
Jejunum (μM)	3.52 ± 0.25	3.52 ± 0.25	0.99
Ileum (μM)	2.36 ± 0.28	2.61 ± 0.21	0.50

Values are presented as mean ± SE.

Table 6 Effect of tributyrin supplementation on concentration of short-chain fatty acids in caecum of 65-week-old laying hens.

Item	Control (0.0%)	Tributyrin 0.05%	P-value
Succinic acid (mmol/L)	9.80 ± 5.17	7.48 ± 2.31	0.27
Lactic acid (mmol/L)	19.68 ± 5.44	19.35 ± 5.26	0.91
Formic acid (mmol/L)	24.33 ± 8.79	23.25 ± 5.40	0.77
Acetic acid (mmol/L)	58.37 ± 16.98	53.67 ± 8.51	0.50
Propionic acid (mmol/L)	19.91 ± 4.33	19.20 ± 5.22	0.77
Butyric acid (mmol/L)	29.77 ± 13.16	32.16 ± 7.32	0.66
Valeric acid (mmol/L)	14.50 ± 8.65	24.08 ± 18.94	0.21

Values are presented as mean ± SD.

Discussion

Although the beneficial effects of organic acids or their salt on the digestibility, nutrient absorption and egg production have been reported (De Freitas et al., 2006; Yesilbag and Colpan, 2006; Hansen et al., 2007; Youssef et al., 2013 and Lee et al., 2015), in the present study, the tributyrin supplementation in the diet did not improve the egg production of the laying hens after the peak period. Swiatkiewicz et al. (2010) and Kaya et al. (2015) also reported that supplementing with an organic acid mixture did not positively affect the productive performance of laying hens. Moreover, Khong et al. (2014) demonstrated that supplementing with sodium butyrate at levels of 0.05, 0.10 and 0.20% of the diet during the late laying cycle (65 weeks old) had no effects on egg production and egg weight. Tributyrin is a neutral short-chain fatty acid triglyceride (Schroder et al., 1998) and it is hydrolyzed by pancreatic and gastric lipases, yielding glycerol and 3 butyrate molecules (Wachtershauser and Stein, 2000), while the free butyric acid molecule is quickly absorbed in the upper small intestine (Bolton and Dewar, 1965). Tributyrin supplementation at 0.05% of the diet may show less acidifying properties throughout the gastrointestinal tract of laying hens and result in no significant effect on the production performance.

Egg quality traits are a very important factor in the economic profitability of egg production (Swiatkiewicz and Arczewska-Wlosek, 2012). Normally, the internal egg quality and egg shell quality decline with the increasing age of hen (Kocevski et al., 2011; Menezes et al., 2012; Roberts et al., 2013) due to the decrease in nutrient absorption for eggshell formation and the increased in egg size and shell surface area (Tumova and Ledvinka, 2009), some dysfunction of the shell gland (Joyner et al., 1987) and the increase in egg weight without increase in the proportion of calcium carbonate deposited in the shells (Khong et al., 2014). However, there have been reported incidences in which the supplementation of organic acids or short chain fatty acids positively improved the eggshell quality (Sengor et al., 2007; Soltan, 2008; Kaya et al., 2014).

The current results are in agreement with those of Sengor et al. (2007), who found that the eggshell breaking strength of old breeder White Bovans hens increased after the inclusion of short-chain fatty acids. Due to the acidifying properties, the supplementation of sodium butyrate also has beneficial effects on egg shell strength, nutrient retention and intestinal villi after the peak period of egg production integrity (Herrera et al., 2009 and Khong et al., 2014). In this study, tributyrin supplementation significantly increased the eggshell thickness and tended to increase the eggshell percentage. However, the improvement in shell strength was not related to the acid-base status in the gastrointestinal tract since there were no acidic properties of tributyrin along the gut.

Increased production of short-chain fatty acids by probiotics or prebiotics increases eggshell quality, proliferation of enterocytes, expression of Ca-binding proteins, Ca absorption, Ca in serum, release of bone modulating factors and overall improvement in the gut health (Hassan et al., 1996; Coudray et al., 2005; Coudray et al., 2006; Scholz-Ahrens et al., 2007; Demigne et al., 2008). The improvement in eggshell quality is closely related to the Ca concentration in the serum (Soltan, 2008). Lutz and Scharrer (1991) reported that Ca was absorbed by the proximal small intestine and by the large intestine. They also found that

butyrate increased Ca absorption due to a Ca-H exchanger in the apical membrane mediating Ca uptake into the epithelial cell in the distal colon of rats. This indicated that tributyrin might improve the mechanism of Ca uptake in the absorptive cells, although the exact mechanism is unclear.

The antimicrobial properties of organic acids have been reported (Russell and Diez-Gonzalez, 1998; Hernandez et al., 2006). In terms of butyric acid, Khong et al. (2014) stated that dietary supplementation of sodium butyrate (0.05, 0.10 and 0.20%) in laying hens during the late laying period linearly increased the *Lactobacillus* spp. population. Moreover, supplementation of butyric acid glyceride at the levels of 0.25 and 0.50% reduced ileal enumerations of total bacteria and *E. coli* in laying hens fed a wheat-based diet (Jahanian and Golshadi, 2015). Nevertheless, in the present study, as tributyrin did not influence the acid-base status of the gastrointestinal tract (Table 4), the populations of *E. coli* and *Lactobacillus* spp. in the caecum were only changed slightly. Moreover, butyric acid that was released from tributyrin may have crossed the membrane of the small and large intestines; consequently, its influence was on cell function rather than the acid-base status in the gut. Accordingly, Barcelo et al. (2000) and Schauber et al. (2003) stated that butyric acid was beneficial in promoting mucosal barrier function by increasing production of mucin and host antimicrobial peptides which act as a barrier to protect the animal from harmful bacteria.

It is generally known that egg production and egg quality decrease as hen age increases. These trends are probably due to the impaired quality of the mucosal cells in the intestine, the decreased length of the intestinal villi (Rahman et al., 2008) and the decrease in the absorption of nutrients. Supplementing the diet of laying hens with sodium butyrate (from 0.03 to 0.05%) during the late laying period (63-73 weeks of age) (Herrera et al., 2009) increased the duodenal villi height and the villi width. Conversely, supplementing the diet of broilers with 0.05% tributyrin reduced the villus height and the ratio of the villus height to crypt depth in the duodenum when compared with the control group (Li et al., 2015). Moreover, Gharib Naseri et al. (2012), Saki et al. (2012) and Kaya et al. (2014) reported that increasing the levels of organic acid in the drinking water of laying hens did not affect intestinal morphology. In the present study, no effect of tributyrin supplementation on the intestinal morphology of laying hens was found. The inconsistent results regarding morphology caused by organic acid supplementation may have been due to the differences in bird age and the growth capacities of the small intestine at the beginning of the supplementation (Kum et al., 2010).

It has been documented that acidic conditions favor the growth of *Lactobacillus* spp., which possibly inhibits the colonization and proliferation of *E. coli* by blocking the sites of adhesion or by producing lactic acid and other metabolites which lower the pH and inhibit *E. coli* (Fuller, 1977). However, the gastrointestinal pH, bacterial population and short chain fatty acid (SCFA) concentrations in the ceacum were not significantly affected by the tributyrin supplementation in this study. Similarly, M'Sadeq et al. (2016) reported that supplementing the diet of broilers with coated sodium butyrate had no effect on the concentration of caecal and ileal SCFAs or intestinal pH. It is assumed that tributyrin may be hydrolyzed by lipase enzyme, yielding glycerol and 3 butyrate molecules (Wachtershauser and Stein, 2000), while the free butyric acid molecule is rapidly absorbed in the upper small intestine (Bolton and Dewar, 1965) and is not effective further down the intestinal tract.

In conclusion, tributyrin supplementation in the diet at 0.05% clearly improved the eggshell thickness of laying hens after peak egg production. However, the production performance, gastrointestinal acid-base, population of *E. coli* and *Lactobacillus* spp. and the SCFA concentration in the caecum were not significantly affected.

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บทคัดย่อ

ผลของการเสริมไตรบิวทีรีนในอาหารต่อผลผลิตไข่ คุณภาพเปลือกไข่ และระบบทางเดินอาหารของไก่ไข่ระยะหลังการให้ไข่สูงสุด

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เพื่อศึกษาผลของการเสริมไตรบิวทีรีนในอาหารต่อประสิทธิภาพการผลิต คุณภาพไข่ และระบบทางเดินอาหารของไก่ไข่ระยะหลังให้ไข่สูงสุด ทำการทดลองในไก่ไข่สายพันธุ์ Lohmann Brown-Classic อายุ 56 สัปดาห์ จำนวน 384 ตัว เป็นระยะเวลา 16 สัปดาห์ แบ่งแม่ไก่ทั้งหมดออกเป็น 2 กลุ่มการทดลอง ๆ ละ 8 ซ้ำ ๆ ละ 24 ตัว แบ่งอาหารทดลองออกเป็น 2 กลุ่ม ได้แก่ 1) ไม่เสริมไตรบิวทีรีนในอาหาร (กลุ่มควบคุม) และ 2) เสริมไตรบิวทีรีน 0.05% ในอาหาร การทดลองพบว่า การเสริมไตรบิวทีรีนในอาหารเพิ่มความหนาของเปลือกไข่อย่างมีนัยสำคัญยิ่ง ($P<0.01$) และมีแนวโน้มทำให้น้ำหนักเปลือกไข่เพิ่มขึ้น ($P=0.07$) อย่างไรก็ตาม ไตรบิวทีรีนไม่มีผลต่อประสิทธิภาพการผลิต สภาวะความเป็นกรด-ด่างในระบบทางเดินอาหาร สัณฐานวิทยาของลำไส้เล็ก ปริมาณเชื้อ *E. coli* และ *Lactobacillus* spp. รวมทั้งความเข้มข้นของกรดไขมันสายสั้นในลำไส้ส่วน caecum ของไก่ไข่ จากผลการทดลองสรุปได้ว่า การเสริมไตรบิวทีรีนในอาหารสามารถเพิ่มความหนาของเปลือกไข่ของไก่ไข่ระยะหลังจากให้ไข่สูงสุดได้ แม้ว่าจะไม่มีผลอย่างมีนัยสำคัญต่อผลผลิตไข่และระบบทางเดินอาหารของไก่ไข่

คำสำคัญ: ผลผลิตไข่ เปลือกไข่ สภาวะในลำไส้ ไก่ไข่ ไตรบิวทีรีน

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