Research Note: Effects of glycerol monolaurate supplementation on egg production, biochemical indices, and gut microbiota of broiler breeders at the late stage of production

Xin Feng,[1,*] Fangang Kong,[*,1] Xian Yan,[†] Liwei Zheng,[*] Qien Qi,[*] Lina Long,[*] Li Gong,[*] Weilong Huang,[‡] and Huihua Zhang[*,2]

[*]*School of Life Science and Engineering, Foshan University, Foshan 528000, China;* [†]*Laboratory of Livestock and Poultry Breeding, Institute of Animal Science, Guangdong Academy of Agricultural Sciences, Guangdong, China; and* [‡]*Kaiping Lvhuang Agriculture and Animal Husbandry Development Co., Ltd, Jiangmen 52931, China*

ABSTRACT The objective of the present study was to determine the effect of glycerol monolaurate (**GML**) supplementation on egg production, biochemical indices, and gut microbiota of broiler breeders at the late stage of production. Total of 180 healthy Qingyuan Partridge broiler breeders were randomly assigned to 2 groups: 1) corn-soybean meal based diet, and 2) basal diet supplemented with 300 mg glycerol monolaurate/kg. Each treatment group had 6 replicates with 15 birds within each replicate. The experiment started at wk 33 and lasted for 8 weeks. Feed conversion rate, egg weight, egg shape index, shell breaking strength, and shell thickness were not different between control and treatment groups. Supplementation of GML significantly decreased the egg breaking rate. All blood chemical indices and antioxidant parameters were not affected by GML except total antioxidant capacity which increased significantly with GML supplementation. Alpha diversity indices (Shannon, Simpson, Chao1, Ace, goods_coverage, and PD_whole tree) were not different between the 2 groups. Composition of cecal microbiota was not affected by GML supplementation except *Euryarchaeota* and *Proteobacteria* at phylum level. Overall, supplementation of glycerol monolaurate at 300 mg/kg level improved eggshell quality but its effect on cecal microbiota composition was limited on broiler breeders at the late stage of production.

Key words: glycerol monolaurate, Qingyuan Partridge chicken, production, microbiota

2021 Poultry Science 100:101386
https://doi.org/10.1016/j.psj.2021.101386

INTRODUCTION

Maintaining a healthy intestinal development is critical to ensure improved growth performance and animal health. Banning of antibiotic additives promote application of various alternatives like organic acids, prebiotics, probiotics, and herbal extracts to improve poultry performanc and product quality. Glycerol monolaurate (**GML**) is formed by a lauric acid attached to the first carbon of the triglycerol via a covalent bond. Compared to lauric acid, GML is biologically more active in neutralizing virus and bacteria. It has been reported to have antiprotozol, antifungal, antibacterial, and antiviral effects and the bacteria did not develop resistance to it.

Chicken health depends on gut condition, nutritional status and immune defense. Supplementation of GML at 4,000 mg/kg increased body weight gain and feed conversion rate of broiler chicks (Mustafa, 2019). It also decreased proinflammatory cytokines and improved total antioxidant capacity. The concentration of serum cholesterol and very low density lipoprotein were decreased too (Mustafa, 2019). GML supplementation (150 mg/kg) improved feed conversion rate and villus height of the small intestine. Elevated apparent metabolic rate of crude protein and meat nutritional compounds (essential Fas, polyunsaturated FAs, protein, lysine, aspartate, glutamate, tyrosine, flavor AAs, and total AAs) of broilers were also observed in that study. Zhao et al. (2019a) reported that supplementation of GML can modify the functional properties of egg white protein by increasing the hardness and improving the thermal stability.

According to our knowledge, the effects of GML on egg quality, biochemical profiles, and gut microbiota of broiler breeders at the late stage of production have not

Received June 13, 2021.
Accepted July 7, 2021.

[1]These authors contribute equally to this work.

[2]Corresponding author: hhzhang2@163.com

been studied yet. The objective of the current study was to investigate how dietary GML supplementation affects egg quality, blood parameters, antioxidant capacity, and cecal microbiota of broiler breeders at the late stage of production.

MATERIALS AND METHODS

Ethics Statement

This experimental protocol was approved by the Ethical Committee and conducted under the supervision of the Institutional Animal Care and Use Committee of Foshan University (Foshan, China).

Experimental Design and Diet

Total of 180 healthy Qingyuan Partridge broilers (33 wk) were randomly assigned to 2 groups: 1) corn-soybean meal based diet, and 2) basal diet supplemented with 300 mg glycerol monolaurate /kg. Each treatment group had 6 replicates with 15 birds in each replicate. The experiment started at wk 33 and lasted for 8 wk. The basal diet was formulated according to the Nutrient Requirements for laying hens (2012). During the study, the birds had free access to feed and drinking water. The room was cleaned and disinfected daily and the house was contronlled at a constant temperature and maintained on a 16 h light regime.

Production Performance and Egg Quality

Feed intake and number of eggs were recorded daily from wk 33 to 40 and feed conversion rate was calculated. The egg breaking rate was calculated based on the number of broken eggs. In the last week of the study, 6 eggs from each replicate (36 eggs per treatment) were randomly selected and egg weight (Egg Analyzer, Orka Food Technology Ltd, Israel), shell breaking stregth (Egg Force Reader, Orka Food Technology Ltd), and shell thickness (Eggshell Thickness Gauge, Orka Food Technology Ltd) were determined. Egg shape index was calculated as the egg width to length ratio. All analysis were conducted by one trained person blind to the treatments.

Blood Sample Collection and Analysis

At the end of the study, one bird was randomly selcted from each replicate. The blood sample was collected from the wing vein and later analyzed for total protein, total cholesteral, albumin, triglyceride, alkaline phosphate, and calcium. Antioxidant parameters including malondialdehyde (**MDA**), total antioxidant capacity (**TAC**), superoxide dismutase (**SOD**), and glutathione peroxidase (**GSH-PX**)were determined according to the instructions provided with the kits (Nanjing Jiancheng Bioengineering Inc., China). The selected chickens were then sacrificed by cervical dislocation and exsanguinated. Intestinal contents from right and left cecum (pooled within broiler) were aseptically collected from each individual broiler and immediately placed into cap vials. The samples were stored at −80°C until further analysis.

Cecal Digesta DNA Extraction and High Throughput Sequencing Analysis

Total genome DNA from cecal digesta was extracted using the Cetyltrimethyl Ammonium Bromide method. Extracted DNA was monitored on 1% agarose gels before being diluted to 1ng/μL to prepare amplicons for high-throughput sequencing. Conventional PCR was used to amplify the V4 regions of the 16S rRNA genes using primers 515F (5'- GTGYCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACNNGGGTATCTAAT-3'). The PCR reaction mix consisted of 15 μL of Phusion High-Fidelity PCR Master Mix (New England Biolabs, Inc., Ipswich, MA), 0.2 μM of forward and reverse primers, and about 10 ng template DNA. Reaction condition consisted of initial denaturation at 98°C for 1 min, followed by 30 cycles of denaturation at 98°C for 10 s, annealing at 50°C for 30 s, elongation at 72°C for 30 s, and a final extension at 72°C for 5 min. The PCR products were mixed with the same volume of 1X loading buffer (contained SYB green) then examined on 2% agarose gene. Only samples with bright strio between 400 and 450 bp were chosen for further analysis. Sequencing libraries were generated using TruSeq DNA PCR-Free sample preparation kit (Illumina, Inc., San Diego, CA) following manufacturer's recommendations and index codes were added. The library quality was assessed on a Qubit @ 2.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA) and Agilent Bioanalyzer 2100 system (Agilent Technologies, Inc., Palo Alto, CA). The bar-coded amplicons were sequenced on an Illumina NovaSeq system and 250 bp paired-end reads were generated.

Paired-end reads were merged using Fast Length Adjustment of Short reads software (V1.2.7) and quality filtering on the raw sequences were conducted on a quality control pipeline using the Quantitative Insight into Microbial Ecology (**QIIME**) tool kit to obtain the high-quality clean reads. Chimera sequences were removed by comparing with the Silva database using UCHIME algorithm. The effective tags were retained for analysis. The obtained high quality reads were assigned to the same operational taxonomic units (**OTUs**) at ≥97% similarity using the QIIME Uclust algorithm. Taxonomic analysis was performed at the phylum and genus levels. OTUs abundance information was normalized and subsequent diversity analysis was performed using the normalized data. Alpha diversity analysis (Shannon, Simpson, Chao1, Ace, and goods-coverage) was conducted to study the complexity of species diversity using QIIME (V1.9.1). Principal coordinate analysis (**PCoA**) was performed to get principal coordinates with bray-

curtis distance algorithm and the data was displayed by WGCNA and ggplot2 packages in R software (V4.0.0).

Statistical Analysis

All data were analyzed using the PROC GLIMMIX procedure of SAS (SAS Institute, Inc., Cary, NC) including treatment as fixed effect in the model. The significance was declared at $P < 0.05$ and trends at $P < 0.1$

RESULTS AND DISCUSSION

Production and Egg Quality Parameters

For broiler breeders, egg production and egg quality are of great economic concerns. Maintaining a high egg shell breaking strength is necessary for lowering economic loss. In current study, supplementation of GML did not affect egg weight and feed conversion ratio from wk 33 to wk 40 ($P > 0.05$; Table 1). The egg breaking rate of the treatment group was significantly lower than that of the control group ($P = 0.03$). Egg shape index, shell breaking strength, and shell thickness were not different between 2 groups ($P > 0.05$). Many factors can affect laying performance, such as genetics, management, nutrition, and environment. GML can be applied as a natural immune enhancer in broiler industry as it can positively impact feed efficiency of broilers and immune response. Supplementation of GML at 300 mg/kg decreased feed conversion rate and increased laying rate and average egg weight. Long-term effects of GML supplementation was also studied and the authors found that GML supplementation of 300 mg GML /kg increased the laying rate and average egg weight (Cai et al., 2020). The feed conversion ratio was also decreased. This was not observed in our study. In that study, the laying hens used were from 58 to 69 wk and the broiler breeders in our study were from 33 to 40 wk. The eggshell thickness and eggshell strength were also improved in that study. Using aged hens Liu et al. (2020a) also demonstrated that GML can improve the laying rate, egg quality including eggshell thickness and strength, and decrease feed conversion rate.

Eggshell thickness and eggshell strength are 2 essential attributes for egg production. Cai et al. (2020) reported supplementing 300 mg GML/kg increased egg shell thickness and eggshell strength. Similarly, eggshell quality was improved with GML supplementation (300 mg/kg; Liu et al., 2020a). However, this was not observed in our study although the GML supplementation did decrease the egg breaking rate. Cai et al. (2020) attributed the improved laying performance and egg quality to the improved absorption capacity shown by increased villus height and crypt depth. Glycerol monolaurate can be broken down into lauric acid and glycerol. It was reported medium-chain fatty acid participate in animal calcium metabolism. The serum calcium concentrations between the 2 groups were not statistically different in our study and this might be the reason that shell strength and shell thickness are not affected.

Blood Biochemical Indices and Antioxidant Parameters

Supplementation of monobutyrin did not affect any of the blood biochemical indexes analyzed other than asparate aminotransferase ($P = 0.09$; Table 1). The GML group tended to have a higher concentration of asparate aminotransferase than the control group. The biochemical blood parameters are beneficial in evaluating the general health condition of the animals and effects of dietary feed additives on animal performance. High alanine aminotransferase (**ALT**) and Aspartate transaminase (**AST**) indicate impaired liver function. Supplementation of GML at 300 mg/kg decreased activities of AST and alkaline phosphatase (Cai et al., 2020). The authors also reported lower concentration of total cholesterol and low-density lipoprotein cholesterol (**LDL-C**) but higher increased concentration of high-density lipoprotein cholesterol (**HDL-C**) indicating improved lipid metabolism. In our study, the treatment group tended to have a lower concentration of AST compared to the control group indicating improved liver function. Surprisingly, high dose of GML (8,000 mg/kg) can cause an adverse effect on the liver (Mustafa, 2019) shown by high levels of ALT and AST. GML supplementation elevated the serum concentration of globulins which are associated to immune response. We only analyzed the total protein concentration which was not affected by GML supplementation. Numerically, the GML group had a higher value than the control group but it was not statistically significant. This was inconsistent with the findings from (Saleh et al., 2021) who reported increased plasma total protein and albumin with 500 mg/kg supplementation.

Regarding to the antioxidant parameters measured, only TAC was affected by glycerol monolaurate supplementation (Table 1). Compared to the control group, supplementation of glycerol monolaurate significantly increased the total antioxidant capacity ($P = 0.04$). Oxidative stress affects animal production and involves in many diseases. The oxidative stress impairs growth and meat quality of broilers. The antioxidant activity of feed additives can be attributed to their effects on gut activity, proinflammatory cytokines, antimicrobial and antiviral effects (Saleh et al., 2021). Zhao et al. (2019b) observed the beneficial effects of GML such as enhanced activity of antioxidant enzymes (SOD and GSH-Px) and decreased MDA content.

Taxonomic Composition of Cecal Microbiota

Rarefaction curve revealed that there was sufficient OTU coverage to describe the bacterial composition of each group. The overall number of OTUs was 1,292 and 952 shared OTUs were detected in both groups. The sequence depth was sufficient enough to capture the

Table 1. Effects of glycerol monolaurate supplementation on production, egg quality, and blood parameters of broiler breeders.

Item	CG	GML	SEM	*P*
Production rate	58.51	62.04	3.241	0.46
Feed conversion rate, g/g	3.17	3.01	0.168	0.54
Egg weight, g	48.44	47.47	0.393	0.11
Egg breaking rate, %	0.87	0.21	0.186	0.03
Egg shape index	1.31	1.29	0.009	0.22
Shell breaking strength, kg/cm^2	3.74	4.04	0.167	0.23
Shell thickness, mm	0.36	0.36	0.005	0.65
Blood biochemical indexes				
Total protein, g/L	8.02	8.86	0.478	0.24
Total cholesterol, mmol/L	6.88	7.67	0.119	0.63
Albumin, g/L	21.47	20.66	2.481	0.82
Triglyceride, mmol/L	15.95	10.32	2.458	0.14
Alkaline phosphatase (ALP), U/L	19.95	12.28	3.231	0.17
Asparate aminotransferase(AST), U/L	16.16	15.46	0.269	0.09
Calcium, mmol/L	3.78	3.81	0.217	0.93
Antioxidant parameters				
MDA, nmol/mL	5.99	4.97	0.919	0.45
TAC, mgprot	5.47	8.08	0.812	0.04
SOD, U/mL	4.73	4.27	0.237	0.19
GSH-PX, U/mL	1415.61	1337.86	90.53	0.55

Abbreviations: CG, control group; GML, glycerol monolaurate; GSH-PX, glutathione peroxidase; MDA, malondialdehyde; SOD, superoxide dismutase; TAC, total antioxidant capacity.

majority of OTUs in the cecal samples. PCoA analysis using the Bray-Curtis similarity method revealed that the first principal component (**PC1**) and the second principal component (**PC2**) explained 26.49 and 14.49% of the variation in microbial diversity, respectively (Figure 1). No distinguishable clustering of samples appeared to be evident between the control and treatment group. Alpha diversity indices including Shannon, Simpson, Chao1, Ace, Goods_coverage, and PD_whole tree were not affected by glycerol monolaurate supplementation (data was not shown). At the phylum level, *Firmicutes* (>39%) and *Bacteroidetes* (>37%) are the first 2 most predominant phylum. Supplementation of glycerol monolaurate tended to increase the

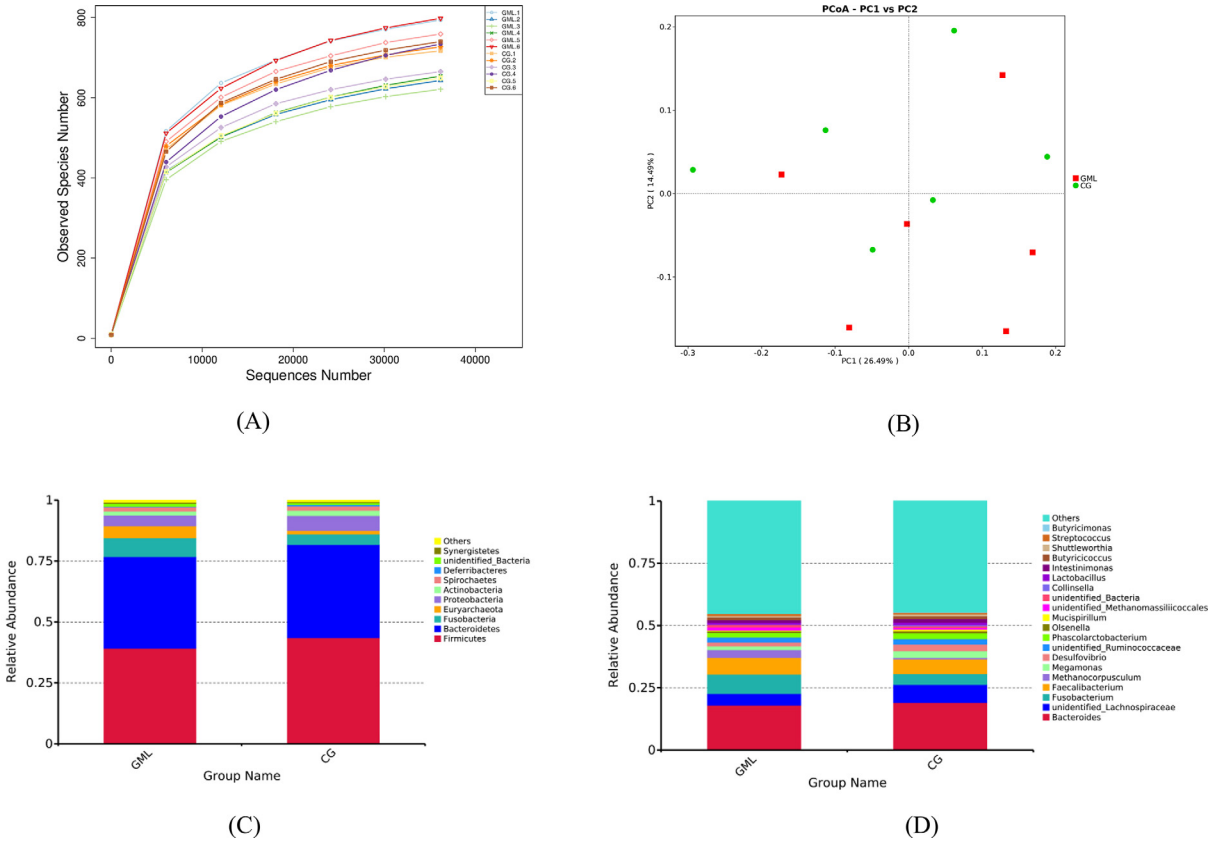

Figure 1. (A) Rarefraction curves of number of operational taxonomic units (OTUs), (B) principle coordinate analysis (PCoA) of the cecal microbiota, (C) phylum-level taxonomic composition of the cecal microbiota, (D) genus-level taxonomic composition of the cecal microbiota. Abbreviations: CG, control group; GML, glycerol monolaurate.

relative abundance of *Euryarchaeota* and significantly decreased the relative abundance of *Proteobacteria*. At the genus level, *Bacteroides* was dominant (>18%), followed by *Lachnospiraceae* (>4%), *Fusobacterium* (>4%), and *Faecalibacterium* (5%). The relative abundance of the rest genera listed were all below 4%. The supplementation of glycerol monolaurate did not affect microbiota composition at genus levels other than *Methanocorpusculum* whereas the GML supplementation tended to increase the relative abundance ($P = 0.07$).

Gut microbiota and their metabolites play important roles in body weight control, metabolic function, and homeostasis. Use of antibiotic drugs on broiler chickens has caused adverse effects on intestinal microbiota and development of antibiotic resistance. Dietary components shape the gut microbiota community resulting in changes in metabolic pathways and metabolites production which ultimately affect host performance (Khan et al., 2020). It has been reported that higher weight gain, feed intake, nutrient utilization as well as better quality of poultry products can be obtained by manipulating gut microbiota (Khan et al., 2020). Liu et al. (2020b) pointed that better performance, intestinal development and muscle composition in broilers were due to its strong antimicrobial capacity. GML was reported to be able to reduce pathogenic bacteria such as *E. coli* and maintain intestinal bacterial homeostasis. Chen et al. (2021) reported 1% GML and a complex disinfectant (0.5 + 0.025% lactic acid) can powerfully inactivate *Samonella* on chicken breast.

GML is stable in high-heat and acidic environment. It is also noncorrosive and volatile in the bird's gut. Results of GML supplementation on gut microbiota are inconsistent. Some reported microbial changes whereas others did not. The inconsistent results could be due to the variation in GML dosage, duration, dietary composition, environmental conditions, animal age, breed, and hygienic conditions. GML supplementation increased abundance of favorable microbiota, particularly acid-producing bacteria on d 28 and reduced opportunistic pathogens such as *Alistipes tidjanibacter* and *Bacteroides dorei* by d 56 (Lan et al., 2021). Liu et al. (2020a) observed altered microbial community and function in aged laying hens after supplementing GML. In their study, the relative abundance of *Fusobacterium* was greatly increased with 300 mg/kg GML. A high level of *Fusobacterium* was not desirable as it is not a common gut microbiota member of chickens. In our study, the relative abundance of *Fusobacterium* also was numerically higher than the control group but not statistically significant. GML also tended to increase the relative abundance of Mucipirillum ($P = 0.07$). The relative abundance of *Tenericutes* was decreased and *Proteobacteria* was dose-dependently elevated (Liu et al., 2020a). In comparison, relative abundance of *Proteobacteria* was decreased in our study. This may be caused by different doses or animal species. Genus *desulfovibrio* are detrimental to host health as it can cause ulcerative colitis by producing toxic sulphide. Supplementing GML at 300 mg/kg numerically decreased the relative abundance of *desulfovibrio* but not statistically different. The high abundance of *Fusobacteria* and *Deferribacteres* were reported helpful for geese to digest the high-grain diet. In our study, the effects of GML supplementation on these 2 phyla were not observed.

In summary, supplementation of glycerol monolaurate decreased the egg breaking rate and increased total antioxidant capacity. It did not affect Alpha diversity indices and composition of cecal microbiota other than *Euryarchaeota* and *Proteobacteria* at phylum level.

ACKNOWLEDGMENTS

The financial support from the Scientific Research Foundation in the Higher Education Institutions of Educational Commission of Guangdong Province (2017GCZX006), Special Foundation for Key Research Area of Educational Commission of Guangdong Province (2019KZDZX2006), Guangdong Province Modern Agriculture Poultry Industry technology system innovation team construction project (2020KJ128), Guangdong Science and Technology Innovation Strategy Special Fund (DZX20192520309), the Special Fund for Scientific Innovation Strategy-Construction of High Level Academy of Agriculture Acience (202107TD), Guangdong Basic and Applied Basic Research Foundation (2019A1515110780), the research start-up fund for Postdoctoral Fellows from Foshan City (BKS209059), and the Scientific research start-up fund for high-level talents of Foshan University (Gg07145) were acknowledged.

DISCLOSURES

The authors declare no conflict of interest.

REFERENCES

Cai, H., M. Liu, J. Li, C. Cai, M. Zhao, and F. Feng. 2020. Effects of long-term dietary glycerol monolaurate supplementation on productivity, egg quality, intestinal mucosal morphology and serum parameters of laying hens. J. Food Sci. Technol. 5:8–17.

Chen, Q., Y. Liu, Z. Zhang, K. Li, B. Liu, and T. Yue. 2021. Bactericidal effect of glycerol monolaurate complex disinfectants on Salmonella of chicken. Int. J. Food Microbiol. 345:109150.

Khan, S., R. J. Moore, D. Stanley, and K. K. Chousalkar. 2020. The gut microbiota of laying hens and its manipulation with prebiotics and probiotics to enhance gut health and food safety. Appl. Environ. Microbiol. 86:e00600–20.

Lan, J., G. Chen, G. Cao, J. Tang, Q. Li, B. Zhang, and C. Yang. 2021. Effects of alpha-glyceryl monolaurate on growth, immune function, volatile fatty acids, and gut microbiota in broiler chickens. Poult. Sci. 100:100875.

Liu, T., C. Li, Y. Li, and F. Feng. 2020a. Glycerol monolaurate enhances reproductive performance, egg quality and albumen amino acids composition in aged hens with gut microbiota alternation. Agriculture 10:250.

Liu, T., J. Tang, and F. Feng. 2020b. Glycerol monolaurate improves performance, intestinal development, and muscle amino acids in yellow-feathered broilers via manipulating gut microbiota. Appl. Microbiol. Biotechnol. 104:10279–10291.

Mustafa, N. G. 2019. Biochemical trails associated with different doses of Alpha-Monolaurin in chicks. Adv. Anim. Vet. Sci. 7: 187–192.

Saleh, A. A., E.-G. B., A. Hassan, N. Badawi, Y. Eid, S. Selim, M. Shukry, and M Dawood. 2021. Effect of dietary inclusion of Alpha-Monolaurin on the growth performance, lipid peroxidation, and immunity response in broilers. Sustainability 13: 5231.

Zhao, M., H. Cai, M. Liu, L. Deng, Y. Li, H. Zhang, and F. Feng. 2019a. Dietary glycerol monolaurate supplementation for the modification of functional properties of egg white protein. J. Sci. Food Agric. 99:3852–3859.

Zhao, M., H. Cai, M. Liu, L. Deng, Y. Li, H. Zhang, and F. Feng. 2019b. Effects of dietary glycerol monolaurate on productive performance, egg quality, serum biochemical indices, and intestinal morphology of laying hens. J. Zhejiang Univ.-SCI B 20:877–890.