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Effect of a mixture of monoglycerides with salinomycin on the growth performance and intestine morphology in broiler chickens

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Abstract. This study aims to interact with the combined influence of monoglyceride supplementation and salinomycin on growth performance and the morphology of the small intestine in broiler chickens. A factorial treatment design was employed in a completely randomized design (CRD). The broiler chickens were randomly divided into four experimental groups, the treatments studied were as follows: 1) Control group (without monoglyceride and salinomycin supplementation), 2) 0.05% monoglyceride supplementation combined without salinomycin, 3) 0.006% salinomycin without monoglyceride supplementation, and 4) 0.05% monoglyceride supplementation combined with 0.006% salinomycin. The study results indicated that the combined supplementation of monoglycerides and salinomycin had an interaction effect on the feed conversion ratio during the first 10 days, villus height: VH in the duodenum, and crypt depth: CD with villus height/crypt depth ratio**:** CD in the jejunum, which were significantly different statistically. Therefore, the supplementation of 0.05% monoglycerides combined with 0.006% salinomycin has an interaction effect on the feed conversion rate to body weight (days 1-10), the increase in VH in the duodenum, and the CD, along with the VH: CD ratio in the jejunum, resulting in improved growth performance and morphology of the proximal small intestine in broiler chickens.

1 Introduction

The poultry industry is a popular source of protein but is now facing the problem of cocidiosiss which causes damage to this industry. Coccidiosis affects productivity and intestinal health. However, the abuse of antibiotic additives induced transferable

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antibiotic resistance in pathogenic bacteria in both birds and human beings.[3] Reducing or replacing the antibiotics in poultry feed has been accepted and promoted worldwide, which urges the scholars and enterprises to find alternative feed additives for the poultry industry.

Salinomycin, an ionophore coccidiostat, is commonly used as a supplement in poultry feed to manage infections caused by *Eimeria spp*. and to prevent necrotic enteritis induced by *Clostridium perfringens* in poultry effect on FCR[6].which aligns with research Conway broiler chickens fed 60 ppm salinomycin for 5 or 6 weeks followed by a withdrawal period of 1 or 2 weeks showed increased feed consumption during the withdrawal period and improved feed conversion ($P < 0.05$) in comparison with unmedicated birds.[2] However, the trend of the poultry market has been changing. For example, American farms raise 60% poultries without using feed containing antibacterial drug. [1] Therefore, preventing cocidiosis without using antibacterial drug in feed is a raising trend and emphasizing the importance of vaccines or mixture of monoglycerides for achieving better effective production is a topic that requires extensive study in the poultry industry.[9]

Combining monoglycerides of butyric-, capric-, and caprylic acid or monoglycerides blend which can be absorbed in small intestine [12] which aligns with the research of Patcharanan et al. [10] who studied the effect of monobutyrin in feed and found that it helps increasing egg production, improving egg quality, and increasing the height of villi in the small intestine which would improve the effectiveness of digesting and absorbing system of poultry after the highest performance of production. Monoglycerides and butyric acid can be absorbed directly in the mid and distal parts of the small intestine. Also, monobutyrin can be broken down into butyric acid in the intestinal mucosa. [15] which aligns with the research of Lin et al. supplement with monoglycerides showed beneficial efficacy on the growth and intestinal function of broilers, demonstrating the potential value of monoglycerides to poultry industry. In terms of dosage, the current trial shows that 3,000 g/t (1−21 d) and 1,000 g/t (22−42 d) monoglycerides without chlortetracycline were the appropriate dietary supplemented rate for broilers. And the mixed use of 500 g/t chlortetracycline and 1,000 g/t monoglycerides was benefit for broilers at 22 to 42 d. [7]

The present study aimed to evaluate the efficacy of monoglycerides combined with salinomycin in broiler diets on growth performance and intestinal morphology in broiler chickens. We hypothesized that the interaction of the mixture of monoglycerides and salinomycin would have positive effects on intestinal histomorphology and improve performance.

2 Materials and methods

2.1 Experimental design and diets

The experiment utilized 1,260 one-day-old ROSS 308 broiler chickens, with a total rearing period of 35 days. A factorial treatment design was employed in a completely randomized design (CRD) for the experiment. All broiler chickens were randomly assigned to four experimental groups, each consisting of six replications with 35 chickens per experimental unit. The treatments under investigation are detailed in Table 1.

Table 1. The level of monoglyceride supplementation combined with salinomycin in broiler chicken feed.

Factor (a)	Factor (b)	Treatment combination
Level of SLM [2]	Level of MGB [3]	a_1b_1 = 0%SLM-0%MGB
a_1 = 0%	b_1 = 0%	a_1b_2 = 0%SLM-0.05%MGB
a_2 = 0.006%	b_2 = 0.05%	a_2b_1 = 0.006%SLM-0%MGB
		a_2b_2 = 0.006%SLM-0.05%MGB

[2]Level of SLM is salinomycin sodium 2 levels according to the FDA guidelines 21 CFR part 558 subpart B Specific New Animal Drugs for Use in Animal Feeds.
[3]Level of MGB is monoglycerides of butyric-, capric- and caprylic acids according to published research of Performance and gut health status of broiler fed diets supplemented with two graded levels of a monoglyceride blend [11]

The experimental feed used the main ingredients of corn and soybean meals, according to the nutritional requirements of ROSS 308 broiler chickens. The feed was divided into three phases: the starter phase (1-10 days), the grower phase (11-24 days), and the finisher phase (25-35 days) (Tables 2 and 3). Throughout the experiment, the broiler chickens had *ad libitum* access to feed and water. The withdrawal period (without salinomycin) lasted for the last 7 days of the experiment.

2.2 Housing management

The experiment was conducted at the Suwannawajakasikij Animal Research Farm, Department of Animal Science, Faculty of Agriculture, Kasetsart University, Bangkok. The number of approval for Animal Care and Use for Scientific Research Kasetsart University is ACKU65-AGR-016. Raising broiler chickens in a floor-released system within a closed housing facility, equipped with an environmental control system utilizing an evaporative cooling system. There are four 50-inch exhaust fans installed at the end of the facility, which operates under a tunnel ventilation system. The pens are partitioned with dimensions of 1.5 meters wide, 3 meters long, and 0.5 meters high, totaling 36 pens. The pen floors are made of concrete and covered with approximately 5 centimeters of rice husk as bedding material. During the first 10 days of the experiment, heat lamps for the chicks were installed in each pen, and feeding is done using yellow trays designed for chicks, while water was provided using chick waterers. From days 11 to 35, feeding was conducted using hanging feeders with a diameter of 10 inches, and an automatic nipple watering system with 7 nipples per pen was implemented.

Table 2. Demonstrate raw materials ratio of each type of feed ingredient in the diets of broiler chickens at different stages.

Ingredients (%)	Starter	Grower	Finisher
Corn	55.10	41.62	40.99
Wheat	-	10.00	15.00
Rice bran defatted	-	5.00	5.00
Corn DDGs	-	8.00	8.00
Soybean oil	2.78	4.97	5.99
Soybean meal 48%	37.63	21.13	15.94
Rape Seed meal	-	4.00	4.00
Feather meal	-	2.00	2.00
L-Lysine hydrochloride	0.19	0.35	0.35
DL-Methionine	0.32	0.24	0.21
L-Threonine	0.09	0.08	0.06
Salt	0.35	0.35	0.35
Calcium carbonate	1.21	1.02	1.08
Mono dicalcium phosphate 21%	1.80	0.73	0.53
Vitamin and Mineral Premix*	0.24	0.24	0.24
Choline chloride 60%	0.28	0.27	0.25
Phytase and Xylanase	-	0.01	0.01
Total	100.00	100.00	100.00

*The vitamin and mineral premix consists of minerals such as copper, iodine, iron, manganese, selenium, and zinc, as well as vitamins including vitamin A, vitamin D, vitamin E, vitamin K, thiamine, riboflavin, niacin, pantothenic acid, pyridoxine, biotin, folic acid, and vitamin B12.

2.3 Growth performance measurements

On days 1, 10, 24, and 35 of the experiment measure according to ROSS 308 guideline, individual body weight of chickens and feed consumptions were weighed using a digital scale (OHAUS Valor 1000 series) to record the initial body weight (IBW) and final weight (FW). This data was used to calculate body weight gain (BWG) and average daily gain (ADG), feed intake (FI), feed conversion ratio (FCR), and mortality (M).

Table 3. Nutrient composition requirements of broilers at starter, grower and finisher

Nutrient	Starter	Grower	Finisher	Units
Metabolize energy for Poultry	3,000	3,100	3,200	Kcal/kg
Protein	23.00	21.50	19.50	%
Fat	5.27	7.81	8.82	%
Fiber	2.56	3.58	3.47	%

Calcium	0.96	0.81	0.78	%
Total Phosphorus	0.72	0.58	0.52	%
Available Phosphorus for Poultry	0.48	0.44	0.39	%
Salt	0.34	0.34	0.34	%
Digestible of amino acids				
Arginine	1.44	1.22	1.08	%
Histidine	0.58	0.50	0.45	%
Isoleucine	0.90	0.79	0.70	%
Leucine	1.81	1.67	1.53	%
Lysine	1.28	1.15	1.02	%
Methionine	0.63	0.53	0.48	%
Methionine and Cysteine	0.95	0.87	0.80	%
Phenylalanine	1.05	0.95	0.86	%
Phenylalanine and Tyrptophan	1.77	1.36	1.20	%
Threonine	0.86	0.77	0.68	%
Tryptophan	0.25	0.21	0.19	%
Valine	0.98	0.92	0.83	%
Choline	1,700	1,600	1,500	mg/kg.

2.4 Intestinal morphology measurements

Samples were collected (one bird representative individual per pen, with six repetitions per treatment) from three section: the duodenum, jejunum, and ileum according to the method of Srinual, Punyatong et al. [14] The samples were processed using an automatic tissue processor, followed by tissue embedding with a tissue embedding machine, and then sectioned using a microtome. The sections were stained with hematoxylin and eosin, and the morphology of the small intestine was recorded using a light microscope at 40x magnification in conjunction with a digital camera. Morphological data of the small intestine were analyzed, including villus height (VH) and crypt depth (CD). The details of the morphological analysis of the small intestine are as follows: villus height was measured from the tip of the villus to the base of the villus, excluding the portion of the measurement for crypt depth, which was measured from the basement membrane to the crypt mouth. The ratio of villus height to crypt depth (VH:CD) was then calculated.
The muscularis mucosae layer, which is thin and located adjacent to the lamina propria, consists of clearly visible smooth muscles and was measured from the upper part of the crypt base.

2.5 Statistical analysis

Data from the growth performance and intestinal morphology recordings were analyzed for variance using a factorial experimental design in a completely randomized trial (factorial experiments in CRD). Data were compared for differences between treatment means using Duncan's multiple range test. The differences between means were set at a significance level of $P < 0.05$ using the SAS® University Edition (Durner 2021) computer program using the following statistical model. [5]

3. Results

3.1 Growth Performance

The supplementation of monoglycerides or salinomycin significantly affected final weight (at 10, 24, and 35 days of age), growth rate (during the ages of 11-24 and 1-35 days), weight gain (during the ages of 1-10, 11-24, and 1-35 days), and feed conversion ratio (during the ages of 1-35 days) with significant differences (p<0.01). The supplementation of salinomycin also had a significant improved on weight gain (during the ages of 25-35 days), feed conversion ratio (during the ages of 11-24 days), and growth rate (during the ages of 1-10 and 25-35 days), with statistically significant differences (p<0.01) as shown in Tables 4 and 5.The supplementation of monoglycerides in conjunction with salinomycin has a significant joint effect on the feed conversion rate to body weight during the period of 1-10 days, with a statistically significant difference (p=0.03)

3.2 Intestinal Morphology

Villus heights in the duodenum were significantly increased in chickens fed with MGBxSLM compared to the control group or those receiving MGB or SLM alone. Additionally, the supplementation of SLM tended to increase crypt depth and the villus-to-crypt ratio (VH:CD). Villus heights in the Jejunum were also enhanced by SLM supplementation. Furthermore, supplementation with MGB, SLM, and the interaction between these groups significantly increased crypt depth and VH:CD in the jejunum. Additionally, supplementation with MGB and SLM increased villus heights in the ileum of broiler intestines, as shown in Table 6.

Table 4. Effect of monoglyceride with salinomycin interaction on growth performance in broiler chickens

Items	MGB		SLM		SEM[1]	*P*-value		
	0.00%	0.05%	0.00%	0.006%		MGB[2]	SLM[3]	MGB ×SLM[4]
Initial Body Weight (g)								
1 d	42.66	42.65	42.66	42.64	0.06	0.91	0.94	0.96
11 d	346.14	356.68	346.59	356.24	1.93	0.005	0.0012	0.29
25 d	1413.80	1454.68	1400.56	1467.92	9.51	0.0005	<0.0001	0.21
Final Body Weight (g)								
10 d	346.14	356.18	346.59	356.24	1.93	0.0005	0.0012	0.29
24 d	1413.80	1454.68	1400.56	1467.92	9.51	0.0005	<0.0001	0.21
35 d	2560.12	2615.31	2531.01	2644.42	16.39	0.01	<0.0001	0.36
Body Weight Gain (g)								
1-10 d	303.48	313.39	303.93	312.94	1.89	0.0012	0.0027	0.20
11-24 d	1067.66	1098.00	1053.97	1111.68	8.16	0.0043	<0.0001	0.30
25-35 d	1146.31	1160.63	1130.45	1176.50	10.86	0.5	0.04	0.77
1-35 d	2517.46	2572.66	2488.35	2601.77	16.37	0.01	<0.0001	0.36

[1] Pooled standard error of the mean.
[2] monoglycerides 0.05% in diet
[3]salinomycin 0.006% in diet
[4]Interaction of monoglycerides and salinomycin.

Table 5. Effect of monoglyceride with salinomycin interaction on growth performance in broiler chickens

Items	MGB		SLM		SEM[1]	*P*-value		
	0.00%	0.05%	0.00%	0.006%		MGB[2]	SLM[3]	MGB× SLM[4]
Average Daily Gain (g/d)								
1-10 d	30.68	31.18	30.38	31.56	0.22	0.12	0.0027	0.06
11-24 d	76.26	78.43	75.28	79.41	0.58	0.0043	<0.0001	0.30
25-35 d	104.21	105.51	102.77	106.96	0.99	0.49	0.04	0.77
1-35 d	74.04	75.67	73.19	76.52	0.48	0.01	<0.01	0.36
Feed Conversion Ratio (g/g)								
1-10 d	1.08	1.06	1.10	1.03	0.01	0.31	0.01	0.03
11-24 d	1.37	1.33	1.39	1.31	0.01	0.06	0.0016	0.21
25-35 d	1.57	1.55	1.58	1.54	0.01	0.40	0.23	0.75
1-35 d	1.43	1.39	1.44	1.38	0.01	0.03	0.0012	0.95
Mortality (%)								
1-10 d	0.25	0.36	0.49	0.00	0.20	0.27	0.81	1.00
11-24 d	0.51	0.25	0.77	0.00	0.21	0.08	0.53	0.53
25-35 d	0.51	0.79	1.04	0.27	0.27	0.17	0.61	0.63
1-35 d	0.97	0.79	1.27	0.49	0.34	0.28	0.80	0.70

[1] Pooled standard error of the mean.
[2] monoglycerides 0.05% in diet
[3]salinomycin 0.006% in diet
[4]Interaction of monoglycerides and salinomycin.

Table 6. Effect of monoglyceride with salinomycin interaction on intestine morphology in broiler chickens at 35 days

Ithems	MGB		SLM		SEM[1]	*P*-value		
	0.00%	0.05%	0.00%	0.006%		MGB[2]	SLM[3]	MGB × SLM[4]
Duodenum								
Villus height (µm)	1380.77	149.01	1376.33	1498.45	33.05	0.02	0.02	0.01
Crypt depth (µm	290.69	350.23	276.66	362.99	16.10	0.01	<0.01	0.06
VH:CD[5]	4.88	4.23	5.03	4.01	0.20	0.04	<0.01	0.17
Jejunum								
Villus height (µm)	647.03	738.00	686.19	704.59	15.27	<0.01	0.42	0.21
Crypt depth (µm	186.06	227.05	192.82	214.32	8.40	<0.01	<0.01	<0.01
VH:CD	3.67	3.10	3.77	3.06	0.17	<0.01	<0.01	<0.01
Ileum								
Villus height (µm)	705.05	592.54	616.30	658.93	15.07	<0.01	<0.01	1
Crypt depth (µm)	185.60	172.98	178.95	180.47	2.65	0.02	0.75	0.64
VH:CD	3.77	3.47	3.64	3.61	0.08	0.09	0.89	0.56

[1] Pooled standard error of the mean.
[2] monoglycerides 0.05% in diet.
[3]salinomycin 0.006% in diet.
[4]Interaction of monoglycerides and salinomycin.
[5]Villus height to Crypt Depth ratio.

4. Discussion

Nowadays, the poultry and swine industries are focusing on developing the use of medium-chain fatty acids and monoglycerides in both *in vitro* and *in vivo* research to study the properties of action to inhibiting viruses and bacteria that can cause diseases in poultry and swine [4] and there are studies from various research to summarize group of active compounds against pathogens and the results from [7] Regarding the effects of combining monoglycerides with chlortetracycline on the growth performance, intestinal morphology, and gut microbiota of the poultry and found that supplementing 500 gram of chlortetracycline per a ton of feed enhances the growth rate of the poultry of 42 days old statistically significantly (p<0.05) comparing to the control group within age of 22-24 days old. The result of supplementing 500 grams of chlortetracycline per ton of feed with monoglycerides 650 or 1,000 gram per ton of feed is show that growth rate is statistically significantly improved (p<0.05) which in line with the study that shows supplementing monoglycerides 0.05% or salinomycin 0.06% in poultry's feed in each age period can improve growth rate poultry.

Supplementing monoglycerides with salinomycin has an influence to improve feed conversion ratio within 1-10 days because supplementation of monoglyceride blends in poultry feed has been found to improve intestinal structure and function to have better immunity protecting digestive system from diseases. Intestine also has better performance which be able to effectively absorb nutrients because is the property of salinomycin that effective prevents some Gram-positive bacteria and inhibits *Clostridium perfringens* that can cause poultry's intestine to inflame which is called necrotic enteritis or NE. However, besides having a better growth rate or higher weight, environment is also a factor to improve performance of monoglycerides [11]

The absorption of monoglycerides occurs directly in the mid and distal parts of the small intestine, where they are then broken down in the mucosa cells in the intestine to butyric acid and be absorbed into blood stream (Because lymphatic system is not developed well in poultry. And then they are metabolized in the liver. [8] For the rest of monoglycerides within distal part of intestine will be converted to butyric acid by microorganism which will be used as energy for the villi cells of the intestine. Therefore, Monoglycerides help improve the morphology of the intestine and enhance the poultry's immune system [13] which consistent with the results of this experiment: combining monoglycerides with salinomycin together will have influence on Villus heigh in Duodenum area to be higher which will improve absorption's efficiency in this area. However, it is highly possible that monoglycerides had some synergy effect with salinomycin on controlling plus-gram bacteria to decline. However, monoglycerides also have features to prevent viruses and support intestine morphology. Therefore, there may be a synergistic effect to improve FCR and intestinal morphology

5. Conclusion

The supplementation of 0.05% monoglycerides combined with 0.006% salinomycin has a synergistic effect on the feed conversion rate to body weight (days 1-10), the height of villi in the duodenum, and crypt depth, along with the VH:CD ratio in the jejunum, resulting in improved growth performance and morphology of the proximal small intestine in broiler chickens.

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