Use of Marine Sulfated Polysaccharide as an alternative to antibiotics in the diet of broilers

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ABSTRACT

The objective of the experiment was to evaluate the efficacy of Marine Sulfated Polysaccharide enhanced by a blend of organic acids (AseaD) as an alternative to colistin in the diet of broilers. A total of two hundred and sixteen one-day-old male chicks (Ross 308, initial body weight: 42.25 ± 0.42 g/bird) were randomly assigned to 1 of 2 treatments each represented with 12 replicate cages of 9 birds. The dietary treatments included (1) basal diet with antibiotic (Control, basal diet + 20 ppm colistin) and (2) basal diet without antibiotic + 0.3% AseaD (AseaD). Birds in the control were fed a basal diet containing colistin from 1 to 28 days of age only. There were no differences in ADG and ADFI between the 2 treatments at any phases or for the overall period ($P > 0.05$). Similarly, no differences in FCR were found during d 1-28 or the overall period ($P > 0.05$). Nevertheless, the FCR of broilers fed AseaD (1.893) was lower than that of broilers fed the control diet (1.991) from 29 to 42 days of age ($P = 0.016$). No differences in the survival rate of birds were found between the 2 treatments ($P > 0.05$). These results confirm the potency of AseaD in broiler diets as a potential alternative to colistin used at a concentration of 20 ppm, with significant benefits and interest during the finishing period when colistin is withdrawn from a diet.

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5. Introduction

Broiler chicks during their early stage of growth are often exposed to multiple potential stressors, such as transportation from hatchery to farm, unfavorable brooding conditions, etc. Besides, their incomplete development of the digestive system and immune functions may make them vulnerable to potentially pathogenic microorganisms leading to depressed growth and high mortality (Adams, 2004; Fascina et al., 2012). Under such conditions, antibiotics are commonly used in poultry diets for the prevention and control of harmful bacteria as well as improvement of growth rate and nutrient digestion. However, the use of antibiotics as growth promoters in animal feeds has been banned in many countries around the world due to a growing public health concern over the evolution of antimicrobial resistance (Castanon, 2007; Singer et al., 2016). Thus, finding alternatives to antibiotics as a feed additive has received much attention from scientists in recent years in

Vietnam and other countries around the world.

It has been shown that organic acids blend and specific seaweed extracts have growth-promoting properties and can be used as substitutes for antibiotics. Panda et al. (2009) showed that 0.4% butyric acid added to a broiler diet improved the FCR while maintaining the growth performance as compared with 0.05% furazolidone. Also, a diet supplemented with a blend of organic acids had a greater growth rate than that supplemented with 0.02% enramycin (Hassan et al., 2010). Beneficial effects of organic acids on the performance of broilers are likely associated with their ability in decreasing enteric pathogens and improving nutrient digestibility (Ghazala et al., 2011; Khan & Iqbal, 2016). However, the effectiveness of organic acids may vary depending on several factors, such as type and dosage of organic acids, buffering capacity of feed ingredients, presence of other antimicrobial compounds and housing conditions. Further, it was shown that marine sulfated polysaccharide (MSP) of *Ulva armoricana* green algae exhibited an antimicrobial activity and stimulated cytokine expression by intestinal epithelial cells (Berri et al., 2016). Tziveleka et al. (2018) also reported that lysozyme complexed with MSP increased its antibacterial activity. Therefore, organic acids combined with MSP would be used as a potential feed additive for improving the health and growth performance of animals, and thereby reducing the use of antibiotics for disease prevention and treatment. The objective of the experiment was to evaluate the efficacy of a Marine Sulfated Polysaccharide enhanced by a blend of organic acids (AseaD) as an alternative to colistin in the diet of broilers.

6. Materials and Methods

6.1. Experimental design, animals and housing

The experiment was conducted using two hundred and sixteen one-day-old male chicks (Ross 308, initial body weight: 42.25 ± 0.42 g/bird. The birds were randomly assigned to 2 dietary treatments in a completely randomized design. The treatments included (1) basal diet with antibiotic (Control) and (2) basal diet without antibiotic + 0.3% AseaD (AseaD). The birds were housed in cages in an open-sided house and each cage measured 1.2 m length x 0.45 m width x 0.4 m height. Each treatment was replicated with 12 cages of 9 birds each. The experiment lasted for 6 weeks.

6.2. Experimental diets and animal feeding

The basal diet was formulated to meet the nutritional requirements of broilers during the experimental period (NRC, 1994). The diets were obtained by adding colistin (20 ppm) or AseaD (0.3%) on top of the basal diet. Birds in the control were fed a basal diet containing colistin from 1 to 28 days of age only. AseaD was included in the AseaD diet and fed throughout the experimental period. AseaD contained a blend of formic acid, citric acid, lactic acid, benzoic acid, sodium butyrate and MSP. This product was provided by Olmix Asialand Co. Ltd, Binh Duong Province, Vietnam. The birds were fed a 3-phase feeding program: phase 1 (1-14 d old), phase 2 (15-28 d old) and phase 3 (29-42 d old). The ingredient composition of the basal diet is presented in Table 4. Diets were in mash form. All birds had free access to water and feed at all times.

6.3. Feed sample analyses

Feed samples were ground to pass through a 1-mm screen before analysis and analyzed according to the standard methods. Diet samples were analyzed for DM (EC 152/2009), CP (AOAC 2001.11), crude fat (TCVN 4331:2001), crude fiber (AOCS Ba-6a-05), ash (EC 152/2009), Ca (AAS08, reference 73/46/EEC), and P (AOAC 965.17). The nutrient analyses were performed by Upscience Vietnam in Binh Duong Province, Vietnam. The analyzed nutrient composition of the basal diet is presented in Table 5.

6.4. Assessment of growth performance and survival rate

The initial body weight of chicks in each cage was recorded at the commencement of the experiment. Subsequent weights of birds and feed disappearance measurements were determined at 14, 28, and 42 days of age. The ADG, ADFI and FCR were calculated on a per-cage basis. The number of dead or removed birds from each cage was recorded daily to calculate the survival rate.

6.5. Statistical analysis

Data were analyzed by an independent Student's t-test to compare the control and treatment groups, using the SAS software (SAS Inst. Inc., Cary, NC). The cage was considered the ex-

Table 4. Ingredient composition of the basal diet (as-fed basis)

Ingredients, g/kg	Days of age		
	1 - 14	15 - 28	29 - 42
Corn, ground	54.70	56.81	59.17
Soybean meal, 46%	35.00	32.80	30.50
Rice bran	4.00	4.00	4.00
Soybean oil	2.40	2.80	3.10
MCP	1.22	1.10	0.96
Limestone	1.70	1.68	1.58
Salt	0.28	0.28	0.28
Vitamin premix[1]	0.10	0.10	0.10
Mineral premix[2]	0.20	0.20	0.20
Phytase	0.02	0.02	0.02
L-Lysine, 78.8%	0.19	0.09	0.00
DL-Methionine, 99%	0.14	0.07	0.04
Pigment	0.05	0.05	0.05

[1]Supplied per kg of feed: vitamin A (10000 IU), vitamin D3 (2000 IU), vitamin E (20 IU), vitamin B2 (5 mg), vitamin B5 (5 mg), vitamin B12 (0.01 mg), niacin (10 mg).
[2]Supplied per kg of feed: Fe (80 mg), Cu (10 mg), Zn (45 mg), Mn (65 mg).

Table 5. Analyzed nutrient composition of the basal diet (as-fed basis)[1]

Items	Days of age		
	1 – 14	15 – 28	29 – 42
ME, kcal/kg[2]	3.000	3.050	3.100
DM, %	88.11	88.45	88.56
Crude protein, %	21.20	20.22	19.28
Crude fat, %	5.32	6.08	7.19
Crude fiber, %	2.54	2.43	2.55
Ash, %	6.25	5.77	5.43
Ca, %	0.99	0.90	0.82
Total phosphorus, %	0.72	0.66	0.62

[1]The analysis was performed by Upscience Vietnam in Binh Duong Province, Vietnam.
[2]Calculated.

perimental unit for live body weight, ADFI, ADG and FCR, whereas one bird was considered the experimental unit for the other parameter. The survival rate between the 2 treatments was compared by the Chi-square test. Treatment effects were considered significant at $P < 0.05$.

7. Results and Discussion

7.1. Growth performance

No differences in the body weight of broilers were found at 1, 14 and 28 days of age between the 2 treatments ($P > 0.05$; Table 6). At d 42, the body weight of birds fed AseaD (2376.1 g/bird) was greater than that of birds fed the control (2355.5 g/bird), but this difference was not statistically significant ($P = 0.598$). There were no differences in ADG and ADFI between the 2 treatments at any phases or for the overall period ($P > 0.05$; Table 7). No differences in FCR were found during 1 to 28 days of age or the overall period ($P > 0.05$). Nevertheless, broilers fed AseaD had a lower FCR than those fed the control from 29 to 42 days of age ($P = 0.016$).

Table 6. Effects of dietary supplementation of AseaD on live body weight of broilers (g/bird)

Age, d	Dietary treatments[1]		SEM	*P*
	Control	AseaD		
1	42.3	42.2	0.123	0.575
14	441.8	435.0	4.297	0.275
28	1306.2	1301.6	12.93	0.803
42	2355.5	2376.1	27.18	0.598

[1]12 replicate cages/treatment and 9 birds/cage.

Table 7. Effects of dietary supplementation of AseaD on growth performance of broilers

Age, d	Dietary treatments[1]		SEM	P
	Control	AseaD		
D 1-14				
ADFI, g	30.92	30.94	0.356	0.968
ADG, g	28.54	27.87	0.303	0.133
FCR	1.084	1.111	0.011	0.104
D 15-28				
ADFI, g	97.27	97.29	1.234	0.992
ADG, g	61.69	61.70	0.779	0.989
FCR	1.578	1.577	0.014	0.985
D 29-42				
ADFI, g	149.17	144.98	1.969	0.146
ADG, g	75.05	76.71	1.335	0.388
FCR	1.991	1.893	0.026	0.016
D 1-42				
ADFI, g	91.99	90.52	0.994	0.304
ADG, g	54.93	54.89	0.702	0.965
FCR	1.676	1.650	0.012	0.163

[1]12 replicate cages/treatment and 9 birds/cage.

For decades, antibiotics have been used in food animal production for disease prevention and growth promotion. However, in recent years, the use of antibiotics as growth promoters has declined due to an increasing concern about antimicrobial resistance in bacteria. Among several non-therapeutic alternatives, organic acids have been shown to perform antimicrobial activities similar to those of antibiotics (Wang et al., 2009). The combination of MSP and organic acids shows an exponential synergy and presents itself as a potent prophylactic strategy to promote animal health, and thereby reducing the need for antibiotics as the MSP was also found to exhibit an antimicrobial activity (Berri et al., 2016).

In this study, broilers fed AseaD had the same growth performance as those fed the control diet (Tables 6 and 7). This indicates that the MSP enhanced by a blend of organic acids is as efficacious as colistin in maintaining the growth rate of broilers. Interestingly, birds fed AseaD had a better FCR than those fed the control diet from 29 to 42 days of age when colistin was withdrawn from the control diet. These results agree with those of previous studies. The improvement in FCR could be due to a better utilization of nutrients as indicated by numerically lower ADFI and greater ADG in broilers fed AseaD. Previous studies showed that organic acids added to a diet for broilers improved both ME and nutrient digestibility (Garcia et al., 2007; Ao et al., 2009; Ghazala et al., 2011).

7.2. Survival rate

There were no differences in the survival rate between the 2 treatments during 1-14 and 15-28 days of age ($P > 0.05$; Figure 2). From 29 to 42 days of age, the survival rate of broilers fed the control diet (98.1%) was also not statistically different ($P = 0.498$) from that of broilers fed AseaD (100.0%). Over a 6-week study, no differences ($P = 0.701$) were found for the survival rate between the 2 treatments, although the survival rate of birds fed AseaD (97.2%) was numerically greater than that of birds fed the control diet (96.3%).

The AseaD-supplemented diet had the same effects on the survival rate as the control diet. This reflects the effectiveness of the MSP combined with a blend of organic acids which is comparable to 0.02% colistin in maintaining the bird health. It was found that a mixture of fumaric acid, calcium formate, calcium propionate, potassium sorbate, calcium butyrate, calcium lactate was more efficient than enramycin in decreasing intestinal *E. coli* and *Salmonella* spp. of broilers (Hassan et al., 2010). Further, the efficacy of AseaD is due to the combination of the MSP properties, enhanced and completed by the organic acid mixture included in the product. It was reported that the MSP of *Ulva armoricana* green algae exhibited an antimicrobial activity

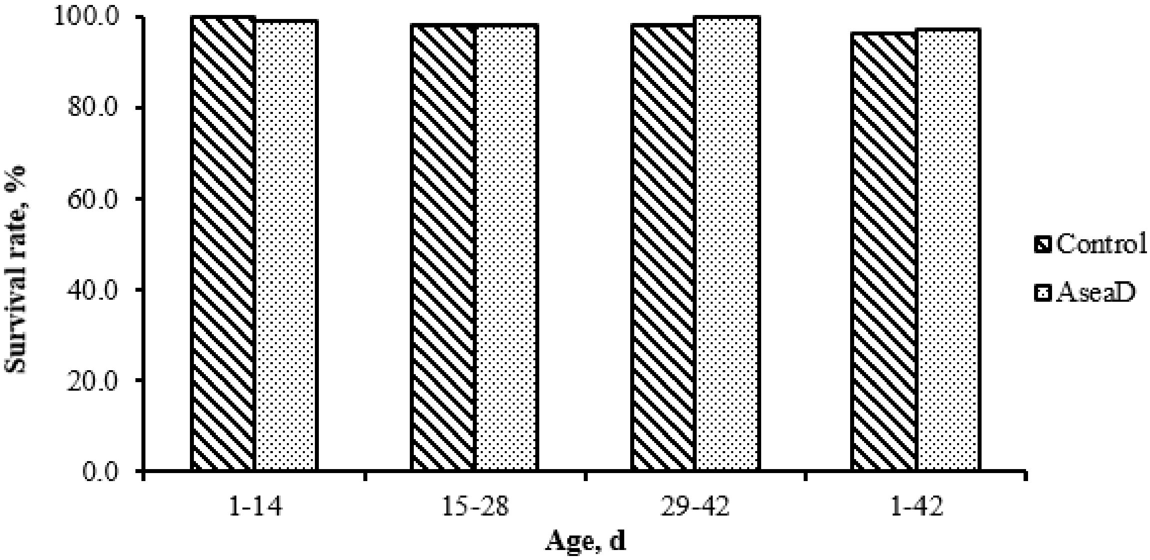

Figure 2. Effects of dietary supplementation of AseaD on the survival rate during the experimental period. There were 108 birds/treatment. No differences were observed for the survival rate during any phases or the overall period between the 2 treatments ($P > 0.05$).

and stimulated cytokine expression by intestinal epithelial cells (Berri et al., 2016). According to Leonard et al. (2011), seaweed extracts (laminarin and fucoidan) added to pig diets improved gut health by reducing the number of colonic *E. coli* and *Enterobacteriaceae*. It has also been proven that certain sulfated polysaccharides from marine algae possess antiviral, antibacterial, antifungal and antioxidant bioactivities (Wang et al., 2011; de Jesus Raposo et al., 2015; Jun et al., 2018). Thus, the suppression of harmful microorganisms by MSP would lead to a better intestinal health, and thereby improving the overall performance and health of birds. At the end of the experiment, both treatments had a high and acceptable bird survival rate (96.3 to 97.2%) like that of previous studies. For example, Rezaei et al. (2018) reported that the survival rate of Ross 308 broilers ranged from 95.5 to 97.3%.

8. Conclusions

Addition of AseaD to a broiler diet resulted in the same growth performance of broilers as a diet supplemented with colistin. The broilers fed the AseaD-supplemented diet had a lower FCR than those fed a diet with colistin from 29 to 42 days of age. These results confirm the potency of AseaD in broiler diets as a potential alternative to colistin used at a concentration of 20 ppm, with significant benefits and interest during the finishing period when colistin is withdrawn from a diet.

Conflicts of interest

The authors declare no conflicts of interest.

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