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A Meta-analysis of Antimicrobial Peptide Effects on Intestinal Bacteria, Immune Response, and Antioxidant Activity of Broilers

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ABSTRACT

This study used a meta-analysis to systematically assess the effect of antimicrobial peptide (AMP) addition on the number of bacteria, immune responses, and antioxidant activity of broilers. The database was compiled from 29 post evaluation articles that were found in search engines consisted of 36 experiments and 111 data. The mixed model method was used to assess the effect of AMP, with AMP addition level as a fixed effect and experiment as a random effect. The fixed effect was tested for linear and quadratic models. The quadratic model was retained when significant at p<0.05 but turned into its corresponding linear model when insignificant. In the starter phase, AMP addition decreased the number of bacteria in the ileum (coliform and total aerobic bacteria (TAB); (p<0.05), the caecum (*Clostridium* spp., *Escherichia coli*, coliform, and lactic acid bacteria (LAB); p<0.05), and excreta (*Clostridium* spp.; p<0.1). Similarly, the number of bacteria also declined in the ileum (*Escherichia coli*, p<0.05; TAB, p<0.1), the caecum (LAB; p<0.1), and excreta (*Clostridium* spp.; p<0.05) of broilers in the finisher phase. There were significant improvements in immune response and antioxidant activity in starter broiler, as indicated by the titer of Newcastle disease (ND) antibody, bursal index, spleen index, and thymus index (p<0.05) due to AMP addition. Variables of immunoglobulin M (IgM), cluster of differentiation 4 (CD4), ND antibody titer, bursal index, spleen index, and thymus index were also significantly increased (p<0.05) while superoxide dismutase activity (SOD activity) tended to increase (p<0.1) in finisher broiler following the AMP addition. In short, AMP addition is able to suppress the number of pathogenic bacteria and increase the immune response and antioxidant activity of broilers.

Keywords: antimicrobial peptide; gut bacteria; immune response; meta-analysis; antioxidant activity

INTRODUCTION

The awareness of the world community on the need for healthy broiler meat has increased recently. Trends in the use of conventional antibiotic growth promoters (AGPs) in broiler diets have become obsolete due to their negative effects to generate resistant pathogenic bacteria and their residual presence in broiler products (Bahar & Ren, 2013; Leeson & Summers, 2009). Accordingly, there is a need to substitute AGP with other compounds, particularly those that originated or are derived from nature like antimicrobial peptides (Gadde *et al.,* 2017; Xiao *et al.,* 2015; Wang *et al.,* 2016). Antimicrobial peptide (AMP) is composed of 4 to 99 amino acids (mostly cationic) that can act as an antifungal, antiviral, antibacterial (i.e., bacteriocidic and bacteriostatic), immunomodulatory, anticancer, antitumor, and antioxidant agent (Bahar & Ren, 2013; Ikeda, 2001; Li *et al.,* 2012; Park & Yoe, 2017a; Park & Yoe, 2017b; Wu *et al.,* 2018; Yi *et al.,* 2014; Zhao *et al.,* 2013). AMP substances can be isolated from animal tissues (e.g., lactoferrin, colostrum, swine antibacterial peptide, and lysozyme), recombinant product (e.g., cecropin AD-asparagine and microcin J25), plants (e.g., thionine and potamic), insects (e.g., defensin-like peptides and diptericin), microbes (e.g., gramicidin and nisin), and amphibians (e.g., magainin) (Bahar & Ren, 2013; Ikeda, 2001; Kim *et al.,* 2005; Li *et al.,* 2017; Park & Yoe, 2017b; Wang *et al.,* 2020; Zhao *et al.,* 2013). The use of AMP as an alternative to substitute conventional AGPs has advantages such as high stability against digestive enzyme degradation, i.e., cysteine-rich peptide (Silva *et al.,* 2000). Also, it tends not to cause resistance effects (due to the β-sheet structure) and has a broad spectrum against various types of pathogens (Bradshaw, 2003; Yi *et al.,* 2014).

Based on *in vitro* studies, the AMP substance, such a defensin, can inhibit gram-positive bacteria (e.g., *Bacillus subtilis* and *Staphylococcus aureus*), *Escherichia coli,* and other types of fungi (Li *et al.,* 2012; Wang *et al.,* 2016). In addition, *in vitro* studies also reported the reduction of oxidative stress as the effect of AMP addition (Ikeda, 2001, Wang *et al*., 2019). Furthermore, *in vivo* study reported the success of AMP to increase productivity through the improvement of the immune response and small intestine ecosystem in the broiler (Choi *et al*., 2013a; Choi *et al*., 2013b; Wang *et al*., 2020). The addition of AMP also shows a positive response to antibody titer (Bai *et al*., 2019). Also, Gong *et al.* (2016) report that lysozyme administration in broilers had no effect on aerobic bacteria, coliforms, and *Clostridium perfringens*. Therefore, this study was conducted to assess the effects of AMP addition on the number of bacteria, immune responses, and antioxidant activity of broiler by integrating data from previously published reports.

MATERIALS AND METHODS

Database Development

A database was developed based on kinds of literature that reported effects of AMP addition on the number of bacteria, immune responses, and antioxidant activity of broiler. The kinds of literature were found in Science Direct and Google Scholar, by using various keywords such as "antimicrobial peptide", "bacterial number", "immune response", "antioxidant activities" and or "broiler". A total of 43 journal articles with digital object identifiers were found. After title and abstract suitability evaluation, 29 articles were entered into the database. The evaluation criteria used were: (1) the article was published in English, (2) the AMP level was determined, and (3) the *in vivo* experiment used a fast-growing broiler. If an article consisted of two or more experiments, the experiments were individually encoded. In total, there were 36 experiments used for meta-analysis that comprised of 111 data points, as depicted in Table 1. This meta-analysis study followed the preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) (Shamseer *et al.,* 2015).

The addition levels of AMP were varied, in a range of 0 (control) to 600 mg kg^{-1} of diet. The used AMP was derived from animal tissue purification (e.g., swine antibacterial peptides, lactoferrin, and bee venom), recombinant products (i.e., microcin J25, AMP-A3, and AMP-P5), and plant-based protein extraction (i.e., bioactive peptides from canola, sesame, and soybean). Broilers were maintained in two phases: starter (ranged between 1-21 days) and finisher (ranged between 22-42 days). Broiler strains used in the meta-analysis were varied, namely Arbor Acres, Cobb 500, Lingnan, Lohmann, Hubbard, and ROSS 308.

The assessed variables were the number of bacteria (e.g. *Clostridium* spp., *Escherichia coli*, coliform, lactic acid bacteria (LAB), and total aerobic bacteria (TAB)), immune responses (e.g., immunoglobulin A (IgA), immunoglobulin M (IgM), cluster of differentiation 3 (CD3), cluster of differentiation 4 (CD4), antibody titer, bursal index, spleen index, and thymus index), and antioxidant activity (e.g., total superoxide dismutase (TSOD), total antioxidant activity (TAA), and superoxide dismutase activity (SOD activity)). Data on growth performance, carcass characteristics, and small intestinal morphology were excluded since they were presented in a separate paper and submitted elsewhere (Sholikin *et al.,* 2020).

Data Analysis

Data analysis was performed in R software version 3.6.3 with additional packages such as "nlme" and "tidyverse" (Bates *et al.,* 2015; Pinheiro *et al.,* 2020; R Core Team, 2020). Linear mixed models (LMM) methodology was performed for the present meta-analysis. The addition level of AMP was fixed effects, while the experiment was random effects (Gałecki & Burzykowski, 2013; Sauvant *et al.,* 2008; St-Pierre, 2001). The mathematical model follows the following equation.

$$Y_{ij} = \beta_0 + \beta_1 Level_{ij} + Experiment_i + Experiment_i Level_{ij} + e_{ij} \quad (1)$$

$$Y_{ij} = \beta_0 + \beta_1 Level_{ij} + \beta_2 Level^2_{ij} + Experiment_i + Experiment_i Level_{ij} + e_{ij} \quad (2)$$

where (1) linear mixed model of the 1st order 1, (2) linear mixed model of the 2nd order, Y_{ij} was dependent variable, β_0 was overall intercept across all studies (fixed effect), β_1 was linear regression coefficient of Y on Level (fixed effect), β_2 was quadratic regression coefficient of Y on Level (fixed effect), $Level_{ij}$ was value of the continuous predictor variable (AMP addition level), $Experiment_i$ was random effect of study i, $Experiment_i Level_{ij}$ was random effect of study i on the regression coefficient of Y on Level in study i, e_{ij} was the unexplained residual error. The p-value, root mean square error (RMSE), and Akaike information criterion (AIC) were used to evaluate the suitability of statistical models (Gałecki & Burzykowski, 2013; Chai *et al.,* 2014). If the p-value was less than or equal to 0.05, the result was significant. In addition, there was a tendency to be significant if only the p-value ranged between 0.05 and 0.1.

RESULTS

The effects of the AMP addition level on the number of bacteria are shown in Table 2. In the ileum, the number of bacteria (coliform and TAB) linearly declined ($p<0.05$) with the increasing AMP level in the starter broiler. Similarly, *Escherichia coli* population linearly decreased ($p<0.05$) due to the AMP addition for the finisher broiler, while the TAB tended to decrease linearly ($p<0.1$). In the caecum of the starter broiler, there was a linear decrease of bacterial numbers, such as *Clostridium* spp., coliform, *Escherichia coli*, and LAB ($p<0.05$) following the AMP addition. Meanwhile, the TAB tended to have a linear increase in finisher broiler ($p<0.1$). In the excreta of the starter broiler, the number of *Clostridium* spp. tended to decline linearly ($p<0.1$). The other bacteria species in the small intestine were not affected by the AMP addition.

Table 1. Literature included in the meta-analysis of antimicrobial peptide addition (mg kg^{-1} of diet) on bacterial population in the small intestine and immune response of broiler

Exp.	Antimicrobial Peptides	Sources	Level	Broiler	Sex	Starter	Finisher	Total	References
1.	Swine antibacterial peptides	Swine intestine	0-200	Arbor Acres	Male	1-21	22-42	1-42	Bao *et al*. (2009)
2.	Swine antibacterial peptides	Swine intestine	0-30	Arbor Acres	Male	1-21	22-42	1-42	
3.	Refined potato protein	*Solanum tuberosum* L.	0-600	ROSS 308	Male	1-21	22-42	1-42	Ohh *et al*. (2009)
4.	AMP-A3	*Helicobacter pylori*	0-90	ROSS 308	-	1-21	22-35	1-35	Choi *et al.* (2013a)
5.	AMP-P5	Analog of Cecropin	0-60	ROSS 308	-	1-21	22-35	1-35	Choi *et al.* (2013b)
6.	Lysozyme	-	0-120	ROSS 308	-	1-21	22-35	1-35	Abdel-Latif *et al.* (2017)
7.	Recombinant plectasin	*Saprophytic ascomycete*	0-200	Arbor Acres	Male	1-21	22-42	1-42	Ma *et al.* (2019)
8.	Camel lactoferrin chimera	-	0-20	Cobb 500	Male	1-10	11-24	1-24	Daneshmand *et al*. (2019a)
9.	Lysozyme	Egg white	0-40	ROSS 308	Male	14-28	29-33	14-33	Torki *et al*. (2018)
10.	Peptide	-	0-250	-	-	1-10	11-28	1-42	Karimzadeh *et al.* (2017a)
11.	Sub lancin	*Bacillus subtilis*	0-11.52	Arbor Acres	-	1-21	22-28	1-28	Wang *et al.* (2015)
12.	Lysozyme	Egg white	0-100	ROSS 308	Male	1-24	25-35	1-35	Gong *et al*. (2017)
13.	Swine antibacterial peptides	Swine intestine	0-0.1	Lohmann	-	-	-	1-42	Wang *et al*. (2009)
14.	Cecropin AD-asparagine	*Hyalophora cecropia*	0-8	Lingnan	Male	14-28	29-42	14-42	Wen & He (2012)
15.	Bee venom	*Apis mellifer*a L.	0-1	Arbor Acres	-	1-28	-	1-28	Han *et al*. (2010)
16.	Glucagon-like peptide 2	-	0-0.33	Arbor Acres	-	1-21	-	1-21	Hu *et al*. (2010)
17.	Glucagon-like peptide 2	-	0-0.33	Arbor Acres	-	1-21	-	1-21	
18.	Lysozyme	-	0-200	Cobb 500	Male	1-28	-	1-28	Zhang *et a*l. (2010)
19.	Lysozyme	-	0-200	Cobb 500	Male	1-28	-	1-28	
20.	Bee venom	*Apis mellifera*	0-0.5	ROSS 308	Male	1-21	-	1-35	Kim *et al*. (2018)
21.	Sesame bioactive peptides	*Sesamum indicum*	0-150	ROSS 308	-	1-24	25-35	1-35	Salavati *et al*. (2019)
22.	Soybean bioactive peptides	Glycine max	0-200	Arbor Acres	-	1-28	29-49	1-49	Jiang *et al*. (2009)
23.	Lysozyme	-	0-40	Arbor Acres	Male	1-14	15-28	1-28	Liu *et al.* (2010)
24.	Lysozyme	-	0-40	Arbor Acres	Male	1-14	15-28	1-28	Liu *et al.* (2010)
25.	Canola bioactive peptides	*Brassica* spp.	0-250	ROSS 308	Male	1-28	29-42	1-42	Karimzadeh *et al.* (2016)
26.	Canola bioactive peptides	*Brassica* spp.	0-250	ROSS 308	Male	1-28	29-42	1-42	Karimzadeh *et al.* (2017b)
27.	Cecropin	Bombyx mori	0-600	Arbor Acres	Mix	1-21	22-42	1-42	Bai *et al*. (2019)
28.	Cecropin	Bombyx mori	0-600	Arbor Acres	Mix	1-21	22-42	1-42	
29.	Cecropin	Bombyx mori	0-600	Arbor Acres	Mix	1-21	22-42	1-42	
30.	Cecropin	Bombyx mori	0-300	Arbor Acres	Mix	1-21	22-42	1-42	
31.	Camel lactoferrin 36	-	0-20	Cobb 500	Male	1-22	-	1-22	Daneshmand *et al*. (2019b)
32.	Bovine lactoferrin	-	0-500	Cobb 500	Male	1-24	25-32	1-32	Geier *et al*. (2011)
33.	Bee venom	*Apis mellifera carnica*	0-1.5	ROSS 308	Mix	1-21	22-42	1-42	Ali & Mohanny (2014)
34.	Bovine lactoferrin	-	0-520	Cobb 500	-	8-28	29-42	8-42	Aguirre *et al*. (2015)
35.	Lactoferrin	-	0-250	Hubbard	Mix	-	-	1-42	Enany *et al*. (2017)
36.	Microcin J25	-	0-1	Arbor Acres	Male	1-21	22-42	1-42	Wang *et al.* (2020)

Note: AMP= Antimicrobial peptide; Exp= Number of experiments.

Table 2. The regression equation of the AMP (mg kg-1 of diet) on the number of bacteria (log10 cfu gram-1) of broiler

No.	Response variable	Model	N	Variable estimates				Model estimates			
				Int.	SE Int.	Slope	SE Slope	p-value	RMSE	AIC[1)]	Trend
Ileum microbes, Starter											
1.	*Clostridium* spp.	L	16	4.2	0.962	-0.004	0.0028	0.198	1.02	49.8	Neg.
2.	Coliform	L	10	4.86	0.663	-0.00489	0.0004	<0.001	0.85	11.1	Neg.
3.	*Escherichia coli*	L	6	4.24	0.269	-0.000987	0.0024	0.715	0.79	9.52	Neg.
4.	LAB	L	6	6.72	0.398	0.00181	0.0094	0.865	1.08	20.1	Pos.
5.	TAB	L	11	7.73	0.45	-0.00416	0.0011	0.011	0.87	17.7	Neg.
Ileum microbes, Finisher											
6.	Coliform	L	6	5.11	0.159	-0.000265	0.0002	0.184	0.88	-2.59	Neg.
7.	*Escherichia coli*	L	8	5.24	0.66	-0.00354	0.0009	0.015	0.97	10.4	Neg.
8.	LAB	L	8	7.49	0.255	-0.000086	0.0034	0.981	1.18	17.8	Neg.
9.	TAB	L	16	7.25	0.656	-0.00293	0.0014	0.059	1.07	42.7	Neg.
Caecum microbes, Starter											
10.	*Clostridium* spp.	L	6	7.24	0.0293	-0.00191	0.0003	0.007	0.85	-18.8	Neg.
11.	Coliform	L	6	5.6	0.791	-0.0038	0.0011	0.038	0.82	5.35	Neg.
12.	*Escherichia coli*	L	18	6.96	0.482	-0.0012	0.0005	0.025	1.26	44	Neg.
13.	LAB	L	15	7.05	0.0786	-0.00111	0.0002	0.002	1.38	3.33	Neg.
14.	TAB	L	13	8.25	0.49	-0.00131	0.0008	0.131	1.07	13.4	Neg.
Caecum microbes, Finisher											
15.	Coliform	L	6	3.62	0.818	-0.000808	0.0011	0.500	0.9	19.7	Neg.
16.	*Escherichia coli*	L	18	7.14	0.667	0.000421	0.0003	0.151	0.91	37.2	Pos.
17.	LAB	L	15	7.57	0.282	0.000403	0.0002	0.083	1.08	15.9	Pos.
18.	TAB	L	12	7.77	0.462	-0.00103	0.0010	0.314	1.24	29.5	Neg.
Excreta microbes, Starter											
19.	*Clostridium* spp.	L	10	7.22	0.307	-0.00472	0.0021	0.070	0.88	14.4	Neg.
20.	Coliform	L	10	6.7	0.317	-0.00351	0.0048	0.489	1.17	24.1	Neg.
21.	TAB	L	14	7.6	0.747	-0.000238	0.0008	0.772	1.39	33.9	Neg.
Excreta microbes, Finisher											
22.	*Clostridium* spp.	L	10	7.72	0.334	-0.00195	0.0012	0.159	1.14	7.1	Neg.
23.	Coliform	L	14	6.296	0.422	-0.000854	0.0009	0.363	1.35	31.8	Neg.
24.	TAB	L	14	7.839	0.522	-0.000371	0.0007	0.599	1.36	27.9	Neg.

Note: AIC= Akaike information criterion; Int.= Intercept; LAB= Lactic acid bacteria; L= Linear; N= Number of data; Neg.= Negative; Pos= Positive; RMSE= Root mean square error; SE= Standard error; TAB= Total aerobic bacteria; [1)]AIC is an estimator of the relative quality of statistical models for a given set of data.

The AMP addition possessed a linear pattern on immune response ($p<0.05$) and antioxidant activity ($p<0.1$) of the broiler (Table 3). In the starter phase, AMP addition linearly increased ($p<0.05$) ND antibody titers and lymphoid organs (i.e., bursal index, spleen index, and thymus index). Similarly, immunoglobulin and complement (IgM; CD4), ND antibody titer, and the spleen organs of the finisher broiler increased in a linear pattern due to AMP addition ($p<0.05$; Table 3), whereas IgA and CD3 were not affected. The effect of AMP addition tended ($p<0.1$) to linearly elevate SOD activity, while TAA was not influenced in finisher broiler. The addition of AMP did not affect TSOD in the starter broiler.

A previous study by Sholikin *et al*. (2020) showed that optimal AMP levels based on feed conversion ratio variables were 337, 359, and 371 mg kg^{-1} in the starter, finisher, and total phases, respectively. The reduction of total *Clostridium* spp. was following equation (3). This was reduced by 8.85% or from 7.24 to 6.60 log10 cfu g^{-1}. The normal rate of *Clostridium* spp. ranged from 7.15 up to 7.27 log10 cfu g^{-1} at the ileum of broiler starter (Choi *et al*., 2013b; Chowdhury *et al*., 2018). Based on equation (4), IgM increased to about 49.33% from 0.58 to 0.87 g L^{-1}. The IgM under normal conditions by Ma *et al*. (2019) is 0.50 g L^{-1}. Based on equation (5), SOD activity increased from 9.35 up to 21.92% inhibition. Karimzadeh *et al*. (2017b) reported that normal broiler SOD activity was 11.40% inhibition.

$$Y_{Clostridiumspp.}=7.24 - 0.00191X_{level}; (p = 0.007) \quad (3)$$

$$Y_{IgM}=0.58 + 0.000797X_{level}; (p = 0.037) \quad (4)$$

$$Y_{SODactivity}= 9.35 + 0.0351X_{level}; (p = 0.01) \quad (5)$$

where (3) *Clostridium* spp. regression equation based on Table 2 row 10, (4) IgM regression equation based on Table 3 row 2, (5) SOD activity regression equation based on Table 3 row 17, Y was dependent variable (variable), and X was independent variable (level of AMP).

DISCUSSION

Effect of AMP Addition on Bacteria Population in The Small Intestine of Broiler

In general, AMP addition is able to reduce the number of pathogenic bacteria in the small intestine of broiler both in starter and finisher phases. Pathogenic bacteria in the small intestine may cause a variety of negative effects, especially tissue damage and also the production of toxic compounds. The accumulation of toxic compounds leads to the emergence of various types of metabolic diseases and may reduce growth performance, nutrient digestibility, and immune response. With regard to the effect of AMP on pathogenic bacteria, the present finding highlights the reduction of the number of *Clostridium* spp. *Clostridium* spp. is a gram-positive bacterium that causes botulism (Chalk *et al.,* 2019; Johnson, 2019). The percentage of *Clostridium* spp. found in the ileum and the caecum of broiler were 9.69% and 39.26% of total bacteria, respectively (Lu *et al*., 2003). Choi *et al.* (2013a) reported the decline of *Clostridium* spp. in the excreta due to AMP-A3 addition (starter and finisher phase). The decline of *Clostridium* spp. is possibly due to the ability of AMP in the form of cecropin-A-maganin-2 (CAMA) to inhibit or even kill gram-positive bacteria (Vizioli *et al.,* 2000). CAMA is composed of an amphipathic terminal base in CA and N-terminal (hydrophobic region) base in MA that both terminals were effective in damaging bacterial cell membranes (Park & Yoe, 2017a; Xiao *et al*., 2015; Yue *et al.,* 2020; Zhang *et al*., 2017).

Escherichia coli and TAB are categorized as coliform group bacteria (Malcolm, 1938). Coliform possesses several characteristics, such as gram negative, lactose base energy source, and aerobic or anaerobic facultative (Malcolm, 1938). Bacteria in this group were able to produce various types of toxic such as indole, skatole, and thionine that may trigger cancer and cause diarrhea (Anabrees *et al*., 2013; Girard & Bee, 2020). The present study confirms the reduction of coliform bacteria numbers like *Escherichia coli* in the ileum and caecum due to AMP addition. This finding was in accordance with previous studies that showed the reduction of coliform bacteria in the ileum after the addition of AMP-P3, lysozyme, and sesame meal bioactive peptide (Choi *et al*., 2013b; Gong *et al*., 2017; Salavati *et al*., 2019). Some types of AMP, such as cecropin (isolated from *Hermetia illucens*) and lysozyme were also effective in inhibiting gram negative bacteria like *Escherichia coli* (Pellegrini *et al*., 1992; Park & Yoe, 2017a). Lysozyme was able to hydrolyze cell walls of both gram-positive and gram-negative bacteria that are composed of peptidoglycan (Ragland & Criss, 2017). The number of TAB decreased in the small intestine and also feces due to the addition of AMP in the form of AMP-A3, AMP-P5, cecropin, and recombinant plectacin (Choi *et al*., 2013b; 2013a; Ma *et al*., 2019; Wen & He, 2012).

In contrast to the present finding, Salavati *et al.* (2019) reported increased LAB number due to lysozyme. Those different findings might be related to the diversity of interactions of AMP against various types of LAB. For instance, lysozyme was reported to have inhibitory activity against several types of LAB like *Lactobacillus brevis* (Tribst *et al*., 2008). Lüders *et al.* (2003) reported that LAB such as *Lactobacillus curvatus* LTH1174 and *Pediococcus acidilactici* LMG 2351 were capable of producing AMPs Curvacin A and Pediocin PA-1.

The reduction of *Clostridium perfringens* population for about 10.9% increased the population of LAB in the ileum for about 2.3% (Askelson *et al*., 2018). Based on 16S rDNA sequences, the number of *Lactobacillus* spp. in the ileum of the broiler was around 67% of total bacteria (Lu *et al*., 2003). *Lactobacillus* spp. could adhere to the small intestine walls and also capable of producing organic acids such as short chain fatty acids (e.g., butyric, propionic, and acetic) and also lactic acid (Rowland *et al*., 2018). These organic acids reduce pH in the small intestine and provide energy available for epithelial cells (Krajmalnik-Brown *et al*., 2012; Shang *et al*., 2018). Energy availability increases cell metabolism so that small intestinal morphology could be maintained.

Table 3. The regression equation of the AMP (mg kg^{-1} of diet) on immune response and antioxidant activities of broiler

No.	Response variable	Unit	Model	N	Variable estimates				Model estimates			
					Int.	SE Int.	Slope	SE Slope	p-value	RMSE	AIC[1)]	Trend
Serum Immunoglobulin and complement, Finisher												
1.	IgA	g/L	L	8	0.657	0.38	6.00E-05	0.0001	0.689	1.06	-12	Pos.
2.	IgM	g/L	L	8	0.58	0.13	0.000797	0.0003	0.037	0.95	-8.15	Pos.
3.	CD3	g/L	L	6	2.49	0.728	0.000775	0.0005	0.204	0.83	11.3	Pos.
4.	CD4	g/L	L	6	0.886	0.639	0.000698	0.0002	0.032	0.83	3.07	Pos.
Newcastle disease antibody titer, Starter[2)]												
5.	Antibody titer	^{2}log(N)	L	13	2.71	0.799	0.00145	0.0003	0.002	1.13	29.4	Pos.
6.	Antibody titer	%	L	11	30.4	1.29	0.0114	0.0028	0.007	1.2	57.9	Pos.
Newcastle disease antibody titer, Finisher[2)]												
7.	Antibody titer	^{2}log(N)	L	17	6.2	0.791	0.00122	0.0006	0.069	1.15	51.4	Pos.
8.	Antibody titer	%	L	11	33.6	1.5	0.0105	0.0033	0.019	1.23	61.3	Pos.
Lymphoid organ index, Starter												
9.	Bursal index		L	11	2.49	0.033	0.000318	0.0001	0.007	1.27	-21.9	Pos.
10.	Spleen index		L	11	0.94	0.0138	0.000151	0.0000	0.004	1.3	-40.9	Pos.
11.	Thymus index		L	11	4.76	0.233	0.00172	0.0005	0.019	1.22	20.8	Pos.
Lymphoid organ index, Finisher												
12.	Bursal index		L	11	1.6	0.0717	0.000509	0.0002	0.032	1.34	-4.31	Pos.
13.	Spleen index		L	11	1.26	0.0145	0.00014	0.0000	0.006	1.27	-40.2	Pos.
14.	Thymus index		L	11	5.07	0.0689	0.000721	0.0002	0.006	1.26	-5.37	Pos.
Antioxidant activity, Starter												
15.	Total superoxide dismutase	U/mg	L	6	43.8	15.8	0.0107	0.0272	0.720	0.84	48	Pos.
Antioxidant activity, Finisher												
16.	Total antioxidant activity	U/mg	L	8	1.81	0.53	0.000782	0.0012	0.538	0.94	8.57	Pos.
17.	Superoxide dismutase	% inhibition	L	5	9.35	2.47	0.0351	0.0150	0.101	1	30.2	Pos.

Note: AIC= Akaike information criterion; CD3= Cluster of differentiation 3; CD4= Cluster of differentiation 4; IgA= Immunoglobulin A; IgM= Immunoglobulin M; Int.= Intercept; L= Linear; N= Number of data; Neg.= Negative; Pos= Positive; RMSE= Root mean square error; SE= standard error; [1)]AIC is an estimator of the relative quality of statistical models for a given set of data; [2)]Antibody titer tested using *Newcastle disease* virus.

In addition, LAB and *Bacillus subtilis* were reported to increase gene expression from mucin that was useful for maintaining mucosa thickness (Aliakbarpour *et al*., 2012).

Effect of AMP Addition on Immune Response and Antioxidant Activity of Broiler

Generally, AMP addition positively affects the broiler immune response such as immunoglobulin, complement, ND antibody titer, and lymphoid organs. Immunoglobulin is the product of B cells (humoral immunity) used to fight antigens (Schat *et al*., 2013). IgA serves an important role in mucosal immunity (in parts of body's secretory organs, respiratory tract, digestive tract, and skin surface) to prevent the attachment of bacteria and viruses to the mucous membrane (Bonner *et al*., 2009; Fagarasan & Honjo, 2003; Macpherson & Slack, 2007; Schat *et al*., 2013). Meanwhile, IgM has a role as a binder of bacteria that attached to the mucosa (Jazayeri *et al*., 2019; Murguia-Favela *et al*., 2017; Sharma, 2017). Complement is a part of cellular immunity and has an important role in T lymphocytes. The function of CD3 is to activate cytotoxic T cells and T helper cells, while CD4 is a receptor of T helper cells that act as a marker (communicating with antigen-presenting cells) (Schat *et al*., 2013). Similar to the finding of Bai *et al.* (2019), the lymphoid organ index was reported to increase in this study. The thymus is the site of differentiation of T lymphocytes, while the bursa of fabricius is a site of maturation of B lymphocytes (Schat *et al*., 2013). In line with the improvement of serum immunoglobulin and complement variables, broilers challenged by the *Newcastle disease* virus and given AMP could increase their antibody titers in both starter and finisher phases. Similar findings by Bai *et al.* (2019) who used cecropin and seaweed powder to increase antibody titers. The increase of IgM, CD4 cell, the lymphoid organ index, and antibody titer have a positive effect on the immune status of broilers. AMP increased innate and adaptive immunity by improving proinflammatory and anti-inflammatory modulation, chemotaxis activity, and direct effects on adaptive immunity (Wang *et al*., 2016). AMP increased the number of T cells and their proliferation products in blood peripherals and also increased IgG, IgM, and IgA in pigs (Ren *et al*., 2015; Yuan *et al*., 2015).

Antioxidant activity of broiler could be assessed based on its SOD activity status. A similar result to the present finding, Karimzadeh *et al.* (2017b) reported the increase of SOD activity in broilers at 42 days by AMP addition in the form of recombinant plectacin. SOD is an enzyme for neutralizing the activity of free radicals such as peroxide and super peroxide (Corpas *et al*., 2006). The proline or arginine-rich AMP (PR-39) proved to inhibit the activity of nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase) from polymorphonuclear leukocytes by blocking the assembly of these enzymes (Ikeda, 2001). The NADPH oxidase itself is the main source of super peroxide. The ability of AMP to suppress free radicals was reported through two main mechanisms, i.e., increasing SOD activity and catalyzing enzymes, and damaging the integrity of NADPH oxidase that is influenced by the activity of N-terminal groups and carboxylic acid groups (Ikeda, 2001; Xiao *et al*., 2015).

CONCLUSION

The present meta-analysis revealed the effect of AMP addition in the form of the decline, not only the number of *Clostridium* spp. at the caecum and excreta in starter broiler but also the number of *Escherichia coli* at the ileum in finisher broiler and at the caecum in starter broiler. Moreover, the number of coliforms at the ileum and the caecum in the starter broiler and TAB at the ileum in the starter and finisher broiler were decreased as the effect of the addition of AMP. The immune response and antioxidant activity of the broiler could also be improved as indicated by the positive responses of serum immunoglobulin M and cluster of differentiation 4, antibody titer, index of lymphoid organs, and SOD activity.

CONFLICT OF INTEREST

Anuraga Jayanegara and Nahrowi serve as editors of the Tropical Animal Science Journal, but have no role in the decision to publish this article. We also declare that there is no conflict of interest with any financial, personal, or other relationships with other people or organization related to the material discussed in the manuscript.

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