Research Article

A Meta-Analysis of the Effect of Antimicrobial Peptide Purity on the Growth Performance, Dry Matter Digestibility, and Intestinal Morphology of Broiler

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Abstract | This meta-analysis aimed to systematically evaluate the effect of the administration of antimicrobial (AMP) both in form of single AMP (SAP) and composite AMP (CAP) on the growth performance, dry matter digestibility, and intestinal morphology of broiler. Data tabulation only involved credible international journals as indicated by Scopus indexed, equipped with doi number, and ranked in the scientific journal rankings cluster. There were 68 experiments with 210 datum collected from 33 literatures. The data were analyzed using a linear mixed model. The differences between the experiments were noted as random effects, while the purity of AMP was determined as fixed effects. The AMP purity significantly (P <0.05) improved the several observed variables, such as body weight, average daily gain, feed conversion ratio, and dry matter digestibility both in the starter period, finisher period, and total period of broiler. It also significantly improved intestinal morphology in the duodenum (alike villus height), jejunum (alike crypt depth), and ileum (like villus height and crypt depth). Compared to CAP, SAP supported better performance on most of observed variables. In short, the AMP could bring positive effect on the growth performance, dry matter digestibility, and intestinal morphology of broiler not only in starter, finisher but also in total of period of broiler. Compared to CAP, the administration of SAP showed a greater performance on broiler.

Keywords | Broiler, Dry matter digestibility, Meta-analysis, Intestinal morphology, Antimicrobial peptide

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INTRODUCTION

The limitation or even prohibition of the use of the antibiotic growth promoter (AGP) has been implemented in the European Union (FAO and IFIF, 2010; Anom, 2019). In similar, Indonesia government have made certain regulation to ban the use of several antibiotic growth promoters. Based on the Minister of Agriculture regulation number 14 on 2017, several AGPs such as avoparcin, beta 1-adrenergic agonist, beta 2-adrenergic agonist, carbadox, carbon tetrachloride, flavomycin, ipronidazole, and roxarsone have been banned as feed additives because of resistant effect (Ministry of Agriculture, 2017). *Escherichia coli* and *Salmonella* spp. reported to resistance to certain antibiotics, namely ciprofloxacin, cephalosporins, ampicillin, and trimoxazole during the period 2004 to 2014 in Tanzania (Gaspary et al., 2017). However, this prohibition caused the disruption on the broiler growth performance usage.

Previous study by Crisol-Martínez et al. (2017) reported that the use of the antibiotic growth promoter, such zinc bacitracin, significantly improved the feed conversion ratio. Therefore, an alternative antibiotic growth promoter is needed especially those with resistant effect, no residues in broiler-derived products and highly effective to kill certain pathogenic microbes that have been resistant to common antibiotics (Yi et al., 2014; Park et al., 2015; Xiao et al., 2015; Gadde et al., 2017).

Antimicrobial peptide (AMP) is a peptide derived from natural materials (vertebrate animal tissue, plants, prokaryotic organisms, and insects) and recombinant products. AMP has a broad spectrum of microbial inhibitory activity (Mylonakis et al., 2016). Based on *in vitro* studies, AMP show various characteristics i.e. resistance to high temperatures (100° C for 15 minutes), show antioxidant, anticancer and germicides activity against various types of pathogenic microbes including gram positive and gram negative bacteria, fungi, yeasts, parasites, and virus (Li et al., 2012; Bahar and Ren, 2013). As a natural product that can synthesize from organic materials, AMP has easily degraded and has not resistant effect (Hassan et al., 2012).

The biological function of AMP as an antimicrobial compound is to inhibit pathogenic microbial activity through the membrane transport system and intercellular activity (Yeaman and Yount, 2003). Inhibition of the membrane transport system is the mechanism of inhibiting cell nutrient transport through the model of barrel-stave, toroidal, carpet, and aggregate channel (Xiao et al., 2015). Inhibition of intercellular activity can be varied in form of the inhibition of DNA, RNA, and protein synthesis, and inducing the formation of reactive oxygen species (ROS). ROS can remove the electron transport mechanism from the mitochondria so that pathogenic bacteria will decrease their growth rate due to lack of energy (Tang et al., 2012). Based on its purity level, AMP is divided into two groups, namely single AMP (SAP) and composite AMP (CAP). The SAP is derived from purification of natural ingredients or recombinant products with a purity level for more than 90 or 95% (Wang et al., 2006; Cao et al., 2012). The SAPs, such as cecropin and lysozyme were reported to have positive effects, such as (i) the improvement of broiler growth performances, (ii) the reduction of the number of pathogenic bacteria (coliform and *Escherichia coli*) in the ileum and cecum, and (iii) the increase of mucosal immunity at starter and finisher period (Zhang et al., 2010; Wen and He, 2012; Choi et al., 2013a; Choi et al., 2013b). In addition, other types of SAP (such as cecropin, defensin, and scorpion toxin) show germicidal properties against antibiotic-resistant bacteria such as *Staphylococcus aureus*, *Salmonella* spp. and *Escherichia coli* (Yeaman and Yount, 2003; Cao et al., 2012; Park and Yoe, 2017a; Park and Yoe, 2017b). In opposite, the CAP is the mixture of several SAPs or derivative products of functional proteins (Karimzadeh et al., 2016). The CAP itself has a purity of less than 50% and sometime its AMP component is not clearly identified. The mixed AMP, such as soybean bioactive peptide and porcine mucosa peptide show a positive effect on broiler growth performance, immunity and gastrointestinal health of broiler (Mateos et al., 2014; Beski et al., 2016; Abdollahi et al., 2017).

Other studies, however, no report to systematically compared the used of SAP and CAP on broiler. Thus, this meta-analysis aimed to comprehensively evaluate the effect of antimicrobial peptide purity on growth performance, dry matter digestibility, and intestinal morphology at starter, finisher and total period of broiler chickens.

MATERIALS AND METHODS

Reference Characteristics

This meta-analysis article used data sources from various regions of the world. Therefore, the regional difference factor was used as a weighting factor in the mathematical model. The condition (e.g., temperature, light, and humidity) of the rearing cage was controlled. The temperature was regulated based on the growing period. Warmer was used in the first week. The references reported that (i) the rearing cage had met the code of conduct for research with animal subjects, (ii) AMP was given to broilers by mixing it into the feed, and (iii) the use of other AGPs in feed was not carried out.

Data Tabulation

Literatures that contained information on the effect of addition of antimicrobial peptide (mg per Kg of feed) on growth performance, dry matter digestibility, and broiler intestinal morphology were determined as targeted literatures. The collection of literature was carried out using the search engines namely "google scholar" and "science direct". The keywords used during the literature searching were "antimicrobial peptide", "cecropin", "lactoferrin", "lysozyme", "broiler", "growth performance", "dry matter digestibility", and "intestinal morphology". Initially, there were 43 literatures that met the criteria to be further evaluated. The criteria used was the abstract of paper should include the AMP dosage and the results in form of broiler growth performance, dry matter digestibility, and intestinal morphology. The evaluation was continued to the entire paper content. Finally, there were 68 experiments that consisted of 210 datums had been collected from 41 literatures.

The result of data collection could be seen in Table 1. Broiler maintenance categories were divided into three periods,

Table 1: Literature involved in the meta-analysis of the effect of antimicrobial peptide purity on the growth performance, dry matter digestibility, and intestinal morphology of broiler

No.	Reference	AMP	Purity	Level[1)]	Breed	Sex	Starter	Finisher	Region	Cage[2)]
1.	Abdel-Latif et al. (2017)	Lisozyme	SAP	0 - 120	ROSS 308	Both	1-21	22-35	Africa	Controlled
2.	Abdollahi et al. (2017)	Soybean bioactive peptide	CAP	0 - 6000	ROSS 308	Male	1-21	-	Australia	Controlled
3.	Abdollahi et al. (2018)	Soybean bioactive peptide	CAP	0 - 6000	ROSS 308	Male	1-21	22-42	Australia	Controlled
4.	Aguirre et al. (2015)	Bovine lactoferrin	SAP	0 - 520	Cobb 500	Both	8-28	29-42	Asia	Controlled
5.	Ali and Mohanny (2014)	Bee venom	SAP	0 - 1.5	ROSS 308	Both	1-21	22-42	Africa	-
6.	Bai et al. (2019)	Cecropin	SAP	0 - 600	Arbor Acres	Both	1-21	22-42	Asia	Controlled
7.	Bao et al. (2009)	Porcine intestinal peptide	CAP	0 - 200	Arbor Acres	Male	1-21	22-42	Asia	Controlled
8.	Beski et al. (2016)	Porcine plasma	CAP	0 - 20000	ROSS 308	Male	1-24	25-35	Australia	Controlled
9.	Choi et al. (2013a)	AMP – A3	SAP	0 - 90	ROSS 308	Both	1-21	22-35	Asia	Controlled
10.	Choi et al. (2013b)	AMP – P5	SAP	0 - 60	ROSS 308	Both	1-21	22-35	Asia	Controlled
11.	Daneshmand et al. (2019)	Lactoferrin	SAP	0 - 20	Cobb 500	Male	1-10	11-24	Asia	Controlled
12.	Daneshmand et al. (2019)	Camel lactoferrin	SAP	0 - 20	Cobb 500	Male	1-22	-	Asia	Controlled
13.	Enany et al. (2017)	Lactoferrin	SAP	0 - 250	Hubbard	Both	-	-	Africa	-
14.	Frikha et al. (2014)	Porcine mucosa peptide	CAP	0 - 75000	ROSS 308	Male	1-15	16-22	Europe	Controlled
15.	Geier et al. (2011)	Bovine lactoferrin	SAP	0 - 500	Cobb 500	Male	1-24	25-32	Australia	Controlled
16.	Gong et al. (2017)	Lisozyme	SAP	0 - 100	ROSS 308	Male	1-24	25-35	America	Controlled
17.	Han et al. (2010)	Bee venom	SAP	0 - 1	Arbor Acres	Both	1-28	-	Asia	Controlled
18.	Hu et al. (2010)	Glucagon-like peptide	SAP	0 - 0.33	Arbor Acres	Both	1-21	-	Asia	Controlled
19.	Humphrey et al. (2002)	Lactoferrin	SAP	0 - 5000	Cobb 500	Male	1-19	-	America	Controlled
20.	Jiang et al. (2009)	Soybean bioactive peptide	SAP	0 - 200	Arbor Acres	Both	1-28	29-49	Asia	Controlled
21.	Józefiak et al. (2018)	Insect peptide	CAP	0 - 2000	ROSS 308	Female	1-21	22-41	Europe	Controlled
22.	Karimzadeh et al. (2016)	Canola bioactive peptide	CAP	0 - 250	ROSS 308	Male	1-28	29-42	Asia	Controlled
23.	Karimzadeh et al. (2017b)	Antimicrobial peptide	CAP	0 - 250	Unknown	Both	1-10	11-28	Asia	Controlled
24.	Karimzadeh et al. (2017b)	Canola bioactive peptide	CAP	0 - 250	ROSS 308	Male	1-28	29-42	Asia	Controlled

25.	Kim et al. (2018)	Bee venom	SAP	0 - 0.5	ROSS 308	Male	1-21	-	Asia	Controlled
26.	King et al. (2005)	Bovine colostrum	CAP	0 - 50000	ROSS 308	Male	1-14	14-35	Australia	Controlled
27.	Liu et al. (2010)	Lisozyme	SAP	0 - 40	Arbor Acres	Male	1-14	15-28	Asia	Controlled
28.	Ma et al. (2020)	Plectasin	SAP	0 - 200	Arbor Acres	Male	1-21	22-42	Asia	Controlled
29.	Mateos et al. (2014)	Porcine mucosa peptide	CAP	0 - 25000	ROSS 308	Both	1-21	22-32	Europe	Controlled
30.	Oblakova et al. (2015)	Natsim	SAP	0 - 300	ROSS 308	Male	1-21	22-49	Europe	Controlled
31.	Ohh et al. (2009)	Potato protein	CAP	0 - 7500	ROSS 308	Male	1-21	22-42	Asia	Controlled
32.	Ohh et al. (2010)	Potato protein	CAP	0 - 7500	ROSS 308	Both	1-21	22-42	Asia	Controlled
33.	Osho et al. (2019)	Soybean bioactive peptide	CAP	0 - 5000	Cobb 500	Male	1-22	-	America	Controlled
34.	Salavati et al. (2020)	Sesame bioactive peptide	SAP	0 - 150	ROSS 308	Both	1-24	25-35	Asia	Controlled
35.	Torki et al. (2018)	Lisozyme	SAP	0 - 40	ROSS 308	Male	14-28	29-33	Europe	Controlled
36.	Wallace and Yang (2010)	Soybean bioactive peptide	CAP	0 - 5000	Unknown	Male	1-21	-	Asia	Controlled
37.	Wang et al. (2009)	Porcine intestinal peptide	CAP	0 - 0.1	Lohman	Both	-	-	Asia	Controlled
38.	Wang et al. (2015)	Sublancin	SAP	0 - 11.52	Arbor Acres	Both	1-21	22-28	Asia	Controlled
39.	Wang et al. (2020)	Microcin J28	SAP	0 - 1	Arbor Acres	Male	1-21	22-42	Asia	Controlled
40.	Wen and He (2012)	Cecropin A	SAP	0 - 8	Lingnan	Male	14-28	29-42	Asia	Controlled
41.	Zhang et al. (2010)	Lisozyme	SAP	0 - 200	Cobb 500	Male	1-28	-	America	Controlled

AMP, Antimicrobial peptide; No., Number of studys; [1)]Unit of antimicrobial peptide is mg per kg of feed; [2)]Controlled environment (e.g., temperature, light, and humidity) of rearing period.

namely: starter period (from the 1st to 21st days) and finisher period (from 21st to 42nd days) and the total period (from the 1st to 42nd days). The observed variables were broiler growth performance, including body weight (g), average daily gain or ADG (g per head per day), average daily feed intake or ADI (g per head per day), feed conversion ratio or FCR, and dry matter digestibility (%). Also, intestinal morphology in the duodenum, jejunum, and ileum such as villus height and crypt depth (μm).

Modelling and Data Analysis

The R software version 3.6.3 with the addition of the "nlme" and "tidyverse" packages was used for modeling and analysis (Pinheiro et al., 2020; R Core Team, 2020). The method used for present meta-analysis was the maxim likelihood model (LMM). The difference in the experiment was determined as random effects and the purity of antimicrobial peptide was noted as fixed effects (St-Pierre, 2001). The statistical model had a P-value and whenever the P-value was less than or equal to 0.05, it meant significant. Also, Akaike information criteria (AIC) and root mean square error (RMSE) were used to evaluate the statistical model (Chai and Draxler, 2014).

Notes: linear mixed model, fixed effect = , random effect = , Y_{ij} = fixed variable, β_0 = the value when the difference in AMP purity intersects the Y-axis for all combinations of random effect, β_1 = specific coefficient of AMP, AMP_{ij} = the differences of AMP purity on random effect, Experiment = experiment number-i, e_{ij} = error model.

Table 2: The effect of antimicrobial peptide purity on the growth performance, dry matter digestibility, and intestinal morphology of broiler

No.	Variable	N	Antimicrobial peptide			P-value
			Control	**SAP**	**CAP**	
1.	Level[1)]		0	249	17,879	
Starter period						
2.	Body weight (gram)	155	782^{a}	792.33^{a}	884^{b}	<0.001
3.	ADG (gram/head/day)	155	36.2^{a}	38.06^{b}	36.6^{b}	<0.001
4.	ADI (gram/head/day)	159	52.5	51.69	53	0.172
5.	Feed conversion ratio	159	1.47^{b}	1.39^{a}	1.48^{b}	<0.001
6.	Dry matter digestibility (%)	31	71.6^{a}	72.81^{ab}	77.8^{b}	0.002
Finisher period						
7.	Body weight (gram)	123	2,221^{a}	2,535^{b}	2,093^{a}	<0.001
8.	ADG (gram/head/day)	123	77^{ab}	86.3^{b}	73.9^{a}	<0.001
9.	ADI (gram/head/day)	123	146^{a}	151.7^{b}	149^{ab}	0.004
10.	Feed conversion ratio	123	1.9^{a}	1.76^{a}	2.02^{b}	<0.001
11.	Dry matter digestibility (%)	19	73.6	75.9	73.6	0.334
Total period						
12.	Body weight (gram)	174	1,816^{a}	2,019^{b}	1,867^{ab}	<0.001
13.	ADG (gram/head/day)	174	55.1^{a}	58.8^{b}	56.2^{a}	<0.001
14.	ADI (gram/head/day)	174	95.6^{b}	93.3^{a}	99.1^{b}	0.001
15.	Feed conversion ratio	174	1.77^{b}	1.58^{a}	1.77^{b}	<0.001
16.	Mortality (%)	23	4.38^{b}	3.21^{ab}	2.95^{a}	0.008
Duodenum						
17.	Villus height (μm)	60	1,120^{a}	1,504^{b}	1,137^{ab}	<0.001
18.	Crypt depth (μm)	51	215	181	211	0.249
Jejunum						
19.	Villus height (μm)	54	938	1,005	1,519	0.224
20.	Crypt depth (μm)	49	197^{ab}	120^{a}	234^{b}	0.036
Ileum						
21.	Villus height (μm)	38	600^{a}	612^{a}	846^{b}	0.007
22.	Crypt depth (μm)	34	159^{b}	111^{a}	150^{ab}	0.002

Feed conversion ratio is the ratio between ADI and ADG; ADI, average daily intake; N, number of data; ADG, average daily gain; Superscript in the same row means a significant difference ($P<0.05$). [1)]Average antimicrobial peptide level added (mg per kg of feed).

RESULT AND DISCUSSION

Although there was difference in term of purity level, both SAP and CAP were able to improve broiler growth performance and dry matter digestibility in all periods as compared to controls (Table 2). In starter period, AMP purity level significantly ($P <0.05$) improved broiler body weight, ADG, FCR, and dry matter digestibility. During starter period, the broiler body weight, FCR, and dry matter digestibility on SAP treatment were significantly ($P <0.05$) lower than those treated with CAP. In finisher period, AMP purity level also significantly ($P <0.05$) improved body weight, ADG, ADI, and FCR. However, dry matter digestibility of SAP and CAP were not significantly different ($P> 0.05$) than control. In the finisher period, the broiler body weight, ADG, and ADI after treated with SAP tended to be higher than that of CAP and the opposite result found in FCR variables, i.e significantly ($P <0.05$) lower. In the total period, the AMP purity level significantly ($P <0.05$) increase broiler body weight, ADG, ADI, and FCR, while the mortality was significantly reduced rather than controls. The broiler body weight and ADG was higher in SAP, while ADI and FCR were significantly lower ($P <0.05$) in SAP than CAP. Broiler intestinal morphology treated with SAP and CAP were better than controls. In duodenum, the SAP treatment produced a higher villus height than controls ($P <0.05$) and CAP. In the jejunum, the crypt depth of SAP treatment was signif

Table 3: The regression equation of the effect of antimicrobial peptide purity on the growth performance, dry matter digestibility, and intestinal morphology of broiler

No.	Variable	N	Variable estimates				Model estimates	
			Int.	SE Int.	Slope	SE Slope	RMSE	AIC[1)]
Starter period								
1.	Body weight (gram)	155	782	41.4	50.2	12.3	0.834	1988
2.	ADG (gram/head/day)	155	36.4	1.51	2.25	0.62	0.835	898
3.	ADI (gram/head/day)	159	52.5	1.99	-0.86	0.59	0.831	943
4.	Feed conversion ratio	159	1.47	0.02	-0.11	0.02	0.836	-370
5.	Dry matter digestibility (%)	31	71.6	1.45	4.28	1.59	0.866	196
Finisher period								
6.	Body weight (gram)	123	2213	67.4	134.3	27.2	0.834	1703
7.	ADG (gram/head/day)	123	76.6	2.32	5.44	1.07	0.834	805
8.	ADI (gram/head/day)	123	146	4.24	2.47	1.52	0.833	922
9.	Feed conversion ratio	123	1.97	0.05	-0.15	0.04	0.835	-161
10.	Dry matter digestibility (%)	19	74.2	0.95	1.97	1.82	0.882	104
Total period								
11.	Body weight (gram)	174	1817	92	138	24.2	0.829	2403
12.	ADG (gram/head/day)	174	55.3	3.01	4.29	0.66	0.830	1099
13.	ADI (gram/head/day)	174	97.8	5.75	1.1	0.71	0.831	1179
14.	Feed conversion ratio	174	1.79	0.04	-0.13	0.02	0.833	-298
15.	Mortality (%)	23	4.38	2.85	-8.2	3.87	0.862	211
Duodenum								
16.	Villus height (μm)	60	1120	95.5	192	64	0.833	890
17.	Crypt depth (μm)	51	216	34.6	-9.63	16.7	0.842	599
Jejunum								
18.	Villus height (μm)	54	938	294	720	788	0.988	906
19.	Crypt depth (μm)	49	198	28.1	-14.5	10.3	0.851	439
Ileum								
20.	Villus height (μm)	38	600	92.8	105	64.9	0.856	609
21.	Crypt depth (μm)	34	159	16.9	-18.7	17.5	0.870	424

ADG, average daily gain; ADI, average daily intake; AIC, akaike information criterion; Int., intercept; N, number of data; RMSE, root mean square errors; SE, standard error; [1)]AIC is an estimator of the accuracy of mathematical model.

icantly (P <0.05) lower than control and CAP. The AMP purity level did not affect the villus height in the jejunum. In the ileum, the villus height and crypt depth on CAP treatment were significantly (P <0.05) better rather than controls. Meanwhile, the SAP treatment had a significantly lower crypt depth (P <0.05) than control.

AMP was reported to have germicidal activity against pathogens originating from bacteria (both gram positive and gram negative bacteria), fungi, yeast, endoparasites, and viruses (Yi et al., 2014; Xiao et al., 2015; Wang et al., 2016; Gadde et al., 2017). Previous study by Choi et al. (2013b) reported that the AMP-P5 (SAP) could increase the ADG and FCR of broiler either in starter and finisher period. Other positive effects were the improvement of broiler growth performance and the decline of pathogenic bacteria in digestive tract as the effect of AMP-A3 (SAP) administration (Choi et al., 2013a). The best dosage of AMP-P5 and AMP-A3 to improve growth performance, nutrient digestibility, intestinal morphology, and coliform reduction were 60 and 90 mg per Kg of feed, respectively. Other studies by Abdel-Latif et al. (2017) and Gong et al. (2017) also displayed similar pattern of finding.

The addition of CAP into the broiler feed could bring positive effect on growth performance and intestinal morphology (King et al., 2005; Wallace and Yang, 2010). Previous study by Ohh et al. (2009) stated that CAP derived from potato contained protein for about 7500 mg per Kg of feed and it resulted the best effect on growth performance and nutrient digestibility at starter and finisher period of broiler. In addition, the CAP derived from porcine mucosa peptide as much as 2500 up to 5000 mg per Kg of feed could increase broiler growth performance during starter period (Frikha et al., 2014).

Based on AMP levels added (Table 2), SAP was lower (e.g., 249 mg per Kg of feed) compared to CAP (e.g., 17,879 mg per Kg of feed). Consequently, SAP was better than CAP and it was highly recommended as an alternative to antibiotic growth promoter. The addition of a low levels of AMP would not affect the nutrient composition of the feed. The finding of this meta-analysis highlighted that the capability of SAP was 50 to 100 times greater than CAP. It might be related to the purity level of AMP used. The purity of SAP was 95% or more (Haeberli et al., 2000; Cao et al., 2012; Wei et al., 2015), while the purity of CAP was only 54.9% (Karimzadeh et al., 2016). Those finding confirmed that the SAP was purer than CAP. Moreover, the CAP also displayed a low antimicrobial activity (Karimzadeh et al., 2016) so that there was a need to increase the CAP dosage to compete with SAP.

There were special techniques required to obtain SAP, such as DNA recombinant, cloning, and staggered isolation using a specific instrument (Park et al., 2015; Park and Yoe, 2017a). Meanwhile, CAP could be produced through hydrolysis process by using protease (Karimzadeh et al., 2017a; Osho et al., 2019). Both SAP and CAP displayed positive effect on growth performance and dry matter digestibility of broiler at starter, finisher and total period. Previous studies confirmed that pure AMP in form of AMP-A3 and AMP-P5 (90 and 60 mg per Kg of feed, respectively) resulted the highest value of villus height and villus height to crypt depth ratio in the duodenum, jejunum and ileum (Choi et al., 2013a; Choi et al., 2013b). In addition, Abdollahi et al. (2017) mixed AMP derived from soybeans as much as 300 mg per Kg of feed also significantly increased the villus height.

Therefore, there was better composition of microbes in the digestive tract, as indicated by the proportion of *Lactobacillus* spp. in the ileum of healthy broilers for about 83% (Apajalahti and Vienola, 2016). In addition, these microbes also produced certain organic acids that could trigger the energy availability to epithelial cells (Krajmalnik-Brown et al., 2012; Shang et al., 2018). Energy availability increased cell metabolism so that intestinal morphology could be maintained (Aliakbarpour et al., 2012). Additionally, the lactic acid bacteria was reported to be able to increase mucosa thickness (Aliakbarpour et al., 2012). Therefore, it was proven that SAP and CAP improved the intestinal morphology of broilers.

CONCLUSION

This meta-analysis concluded that the addition of AMP could improve the growth performance of broiler chickens as indicated by body weight, average daily gain, dry matter digestibility and intestinal morphology both in the starter period, finisher period, and total period of broiler. AMP constantly reduced FCR value in starter and finisher periods. Compared to CAP, the administration of SAP showed a greater performance on broiler.

CONFLICT OF INTEREST

We declare no competing interests.

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AUTHORS CONTRIBUTION

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REFERENCES

- Abdel-Latif MA, El-Far AH, Elbestawy AR, Ghanem R, Mousa SA, Abd El-Hamid HS (2017). Exogenous dietary lysozyme improves the growth performance and gut microbiota in broiler chickens targeting the antioxidant and non-specific immunity mRNA expression. In: Kunze, G. (Ed.), PLOS ONE. 12(10): e0185153. https://doi.org/10.1371/journal.pone.0185153.
- Abdollahi MR, Zaefarian F, Gu Y, Xiao W, Jia J, Ravindran V (2018). Influence of soybean bioactive peptides on performance, foot pad lesions and carcass characteristics in broilers. J. Appl. Anim. Nutrit. 6: 1–7. https://doi.org/10.1017/JAN.2018.1.
- Abdollahi MR, Zaefarian F, Gu Y, Xiao W, Jia J, Ravindran V (2017). Influence of soybean bioactive peptides on growth performance, nutrient utilisation, digestive tract development and intestinal histology in broilers. J. Appl. Anim. Nutrit. 5: 1–7. https://doi.org/10.1017/JAN.2017.6.
- Aguirre ATA, Acda SP, Angeles AA, Oliveros MCR, Merca FE, Cruz FA (2015). Effect of bovine lactoferrin on growth performance and intestinal histologic features of broilers. Philipp. J. Vet. Anim. Sci. 41(1): 12–20.
- Ali A, Mohanny K (2014). Effect of injection with bee venom extract on productive performance and immune response of broiler chicks. J. Anim. Poult. Prod. 5(5): 237–246. https://doi.org/10.21608/jappmu.2014.69561.
- Aliakbarpour HR, Chamani M, Rahimi G, Sadeghi AA, Qujeq D (2012). The *Bacillus subtilis* and lactic acid bacteria probiotics influences intestinal mucin gene expression,

histomorphology and growth performance in broilers. Asian-Australasian J. Anim. Sci. 25(9): 1285–1293. https://doi.org/10.5713/ajas.2012.12110.

- Anom (2019). The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2017. EFSA J. 17(2). https://doi.org/10.2903/j.efsa.2019.5598.
- Apajalahti J, Vienola K (2016). Interaction between chicken intestinal microbiota and protein digestion. Anim. Feed Sci. Technol. 221: 323–330. https://doi.org/10.1016/j.anifeedsci.2016.05.004.
- Bahar A, Ren D (2013). Antimicrobial peptides. Pharmaceuticals. 6(12): 1543–1575. https://doi.org/10.3390/ph6121543.
- Bai J, Wang R, Yan L, Feng J (2019). Co-supplementation of dietary seaweed powder and antibacterial peptides improves broiler growth performance and immune function. Brazilian J. Poult. Sci. 21(2). https://doi.org/10.1590/1806-9061-2018-0826.
- Bao H, She R, Liu T, Zhang Y, Peng KS, Luo D, Yue Z, Ding Y, Hu Y, Liu W, Zhai L (2009). Effects of pig antibacterial peptides on growth performance and intestine mucosal immune of broiler chickens. Poult. Sci. 88(2): 291–297. https://doi.org/10.3382/ps.2008-00330.
- Beski SSM, Swick RA, Iji PA (2016). Effect of dietary inclusion of spray-dried porcine plasma on performance, some physiological and immunological response of broiler chickens challenged with Salmonella sofia. J. Anim. Physiol. Anim. Nutrit. 100(5): 957–966. https://doi.org/10.1111/jpn.12414.
- Cao L, Li Z, Zhang R, Wu Y, Li W, Cao Z (2012). StCT2, a new antibacterial peptide characterized from the venom of the scorpion *Scorpiops tibetanus*. Peptides. 36(2): 213–220. https://doi.org/10.1016/j.peptides.2012.04.010.
- Chai T, Draxler RR (2014). Root mean square error (RMSE) or mean absolute error (MAE)? – Arguments against avoiding RMSE in the literature. Geoscient. Model Develop. 7(3): 1247–1250. https://doi.org/10.5194/gmd-7-1247-2014.
- Choi SC, Ingale SL, Kim JS, Park YK, Kwon IK, Chae BJ (2013a). An antimicrobial peptide-A3: effects on growth performance, nutrient retention, intestinal and faecal microflora and intestinal morphology of broilers. British Poult. Sci. 54(6): 738–746. https://doi.org/10.1080/00071668.2013.838746.
- Choi SC, Ingale SL, Kim JS, Park YK, Kwon IK, Chae BJ (2013b). Effects of dietary supplementation with an antimicrobial peptide-P5 on growth performance, nutrient retention, excreta and intestinal microflora and intestinal morphology of broilers. Anim. Feed Sci. Technol. 185: 78–84. https://doi.org/10.1016/j.anifeedsci.2013.07.005.
- Crisol-Martínez E, Stanley D, Geier MS, Hughes RJ, Moore RJ (2017). Understanding the mechanisms of zinc bacitracin and avilamycin on animal production: linking gut microbiota and growth performance in chickens. Appl. Microbiol. Biotechnol. 101(11): 4547–4559. https://doi.org/10.1007/s00253-017-8193-9.
- Daneshmand A, Kermanshahi H, Sekhavati MHH, Javadmanesh A, Ahmadian M, Alizadeh M, Aldavoodi A (2019). Effects of cLFchimera, a recombinant antimicrobial peptide, on intestinal morphology, microbiota, and gene expression of immune cells and tight junctions in broiler chickens challenged with *C. perfringens*. BioRxiv. https://doi.org/10.1101/871467.
- Enany M, El Gammal AEA, Solimane R, El Sissi A, Hebashy A (2017). Evaluation of lactoferrin immunomodulatory effect on the immune response of broiler chickens. Suez Canal Vet. Med. JournalAK/S. SCVMJ. 22(1): 135–146. https://doi.org/10.21608/scvmj.2017.62452.
- FAO, IFIF (2010). Good practices for the feed industry - Implementing the Codex Alimentarius Code of Practice on Good Animal Feeding. FAO Animal Production and Health Manual No. 9, Rome.
- Frikha M, Mohiti-Asli M, Chetrit C, Mateos GG (2014). Hydrolyzed porcine mucosa in broiler diets: Effects on growth performance, nutrient retention, and histomorphology of the small intestine. Poult. Sci. 93(2): 400–411. https://doi.org/10.3382/ps.2013-03376.
- Gadde U, Kim WH, Oh ST, Lillehoj HS (2017). Alternatives to antibiotics for maximizing growth performance and feed efficiency in poultry: a review. Anim. Health Res. Rev. 18(1): 26–45. https://doi.org/10.1017/S1466252316000207.
- Gaspary OM, Murugan S, Joram B, Bernadether TR, Douglas RC (2017). A systematic review of antibiotic-resistant *Escherichia coli* and *Salmonella* data obtained from Tanzanian healthcare settings (2004-2014). African J. Microbiol. Res. 11(2): 45–54. https://doi.org/10.5897/AJMR2016.8282.
- Geier MS, Torok VA, Guo P, Allison GE, Boulianne M, Janardhana V, Bean AGD, Hughes RJ (2011). The effects of lactoferrin on the intestinal environment of broiler chickens. Brit. Poult. Sci. 52(5): 564–572. https://doi.org/10.1080/00071668.2011.607429.
- Gong M, Anderson D, Rathgeber B, MacIsaac J (2017). The effect of dietary lysozyme with EDTA on growth performance and intestinal microbiota of broiler chickens in each period of the growth cycle. J. Appl. Poult. Res. 26(1): 1–8. https://doi.org/10.3382/japr/pfw041.
- Haeberli S, Kuhn-Nentwig L, Schaller J, Nentwig W (2000). Characterisation of antibacterial activity of peptides isolated from the venom of the spider *Cupiennius salei* (Araneae: Ctenidae). Toxicon. 38(3): 373–380. https://doi.org/10.1016/S0041-0101(99)00167-1.
- Han SM, Lee KG, Yeo JH, Oh BY, Kim BS, Lee W, Baek HJ, Kim ST, Hwang SJ, Pak SC (2010). Effects of honeybee venom supplementation in drinking water on growth performance of broiler chickens. Poult. Sci. 89(11): 2396–2400. https://doi.org/10.3382/ps.2010-00915.
- Hu XF, Guo YM, Huang BY, Bun S, Zhang LB, Li JH, Liu D, Long FY, Yang X, Jiao P (2010). The effect of glucagon-like peptide 2 injection on performance, small intestinal morphology, and nutrient transporter expression of stressed broiler chickens. Poult. Sci. 89(9): 1967–1974. https://doi.org/10.3382/ps.2009-00547.
- Humphrey BD, Huang N, Klasing KC (2002). Rice expressing lactoferrin and lysozyme has antibiotic-like properties when fed to chicks. J. Nutrit. 132(6): 1214–1218. https://doi.org/10.1093/jn/132.6.1214.
- Jiang YB, Yin QQ, Yang YR (2009). Effect of soybean peptides on growth performance, intestinal structure and mucosal immunity of broilers. J. Anim. Physiol. Anim. Nutrit. 93(6): 754–760. https://doi.org/10.1111/j.1439-0396.2008.00864.x.
- Józefiak A, Kierończyk B, Rawski M, Mazurkiewicz J, Benzertiha A, Gobbi P, Nogales-Merida S, Świątkiewicz S, Józefiak D (2018). Full-fat insect meals as feed additive – the effect on broiler chicken growth performance and gastrointestinal tract microbiota. J. Anim. Feed Sci. 27(2): 131–139. https://doi.org/10.22358/jafs/91967/2018.

- Karimzadeh S, Rezaei M, Teimouri-Yansari A (2017a). Effect of canola peptides, antibiotic, probiotic and prebiotic on performance, digestive enzymes activity and some ileal aerobic bacteria in broiler chicks. Iranian J. Anim. Sci. 48(7): 129–139. https://doi.org/10.22059/ijas.2017.221313.653481.
- Karimzadeh S, Rezaei M, Teimouri-Yansari A (2016). Effects of canola bioactive peptides on performance, digestive enzyme activities, nutrient digestibility, intestinal morphology and gut microflora in broiler chickens. Poult. Sci. J. 4(1): 27–36. https://doi.org/10.22069/PSJ.2016.2969.
- Karimzadeh S, Rezaei M, Yansari AT (2017b). Effects of different levels of canola meal peptides on growth performance and blood metabolites in broiler chickens. Livest. Sci. 203: 37–40. https://doi.org/10.1016/j.livsci.2017.06.013.
- Ministry of Agriculture (2017). Regulation of the Minister of Agriculture of the Republic of Indonesia Number 14/ Permentan/PK.350/5/2017 concerning the Classification of Veterinary Drugs.
- Kim DH, Han SM, Keum MC, Lee S, An BK, Lee S-R, Lee K-W (2018). Evaluation of bee venom as a novel feed additive in fast-growing broilers. Brit. Poult. Sci. 59(4): 435–442. https://doi.org/10.1080/00071668.2018.1476675.
- King MR, Ravindran V, Morel PCH, Thomas D V., Birtles MJ, Pluske JR (2005). Effects of spray-dried colostrum and plasmas on the performance and gut morphology of broiler chickens. Australian J. Agric. Res. 56(8): 811. https://doi.org/10.1071/AR04324.
- Krajmalnik-Brown R, Ilhan Z, Kang D, DiBaise JK (2012). Effects of gut microbes on nutrient absorption and energy regulation. Nutrit. Clin. Pract. 27(2): 201–214. https://doi.org/10.1177/0884533611436116.
- Li Y, Xiang Q, Zhang Q, Huang Y, Su Z (2012). Overview on the recent study of antimicrobial peptides: Origins, functions, relative mechanisms and application. Peptides. 37(2): 207–215. https://doi.org/10.1016/j.peptides.2012.07.001.
- Liu D, Guo Y, Wang Z, Yuan J (2010). Exogenous lysozyme influences *Clostridium perfringens* colonization and intestinal barrier function in broiler chickens. Avian Pathol. 39(1): 17–24. https://doi.org/10.1080/03079450903447404.
- Ma JL, Zhao Li Hua, Sun DD, Zhang J, Guo YP, Zhang ZQ, Ma QG, Ji C, Zhao Li Hong (2020). Effects of dietary supplementation of recombinant plectasin on growth performance, intestinal health and innate immunity response in broilers. Probiot. Antimicrob. Proteins. 12(1): 214–223. https://doi.org/10.1007/s12602-019-9515-2.
- Mateos GG, Mohiti-Asli M, Borda E, Mirzaie S, Frikha M (2014). Effect of inclusion of porcine mucosa hydrolysate in diets varying in lysine content on growth performance and ileal histomorphology of broilers. Anim. Feed Sci. Technol. 187: 53–60. https://doi.org/10.1016/j.anifeedsci.2013.09.013.
- Mylonakis E, Podsiadlowski L, Muhammed M, Vilcinskas A (2016). Diversity, evolution and medical applications of insect antimicrobial peptides. Philosophical Transactions of the Royal Society B: Biolog. Sci. 371: 1–11. https://doi.org/10.1098/rstb.2015.0290.
- Oblakova M, Sotirov L, Lalev M, Hristakieva P, Mincheva N, Ivanova I, Bozakova N, Koynarski T (2015). Growth performance and patural humoral immune status in broiler chickens treated with the immunomodulator natstim. Int. J. Curr. Microbiol. App. Sci. 4(11): 1–7.
- Ohh SH, Shinde PL, Choi JY, Jin Z, Hahn TW, Lim HT, Kim GY, Park YK, Hahm KS, Chae BJ (2010). Effects of potato (*Solanum tuberosum* l. cv. golden valley) protein on performance, nutrient metabolizability, and cecal microflora in broilers. Archiv. Fur. Geflugelkunde. 74(1): 30–35.
- Ohh SH, Shinde PL, Jin Z, Choi JY, Hahn T-W, Lim HT, Kim GY, Park Y, Hahm K-S, Chae BJ (2009). Potato (*Solanum tuberosum* L. cv. Gogu valley) protein as an antimicrobial agent in the diets of broilers. Poult. Sci. 88(6): 1227–1234. https://doi.org/10.3382/ps.2008-00491.
- Osho SO, Xiao WW, Adeola O (2019). Response of broiler chickens to dietary soybean bioactive peptide and coccidia challenge. Poult. Sci. 98(11): 5669–5678. https://doi.org/10.3382/ps/pez346.
- Park S, Kim J, Yoe SM (2015). Purification and characterization of a novel antibacterial peptide from black soldier fly (*Hermetia illucens*) larvae. Develop. Comparat. Immunol. 52(1): 98–106. https://doi.org/10.1016/j.dci.2015.04.018.
- Park S, Yoe SM (2017a). Defensin-like peptide3 from black solder fly: Identification, characterization, and key amino acids for anti-Gram-negative bacteria. Entomolog. Res. 47(1): 41–47. https://doi.org/10.1111/1748-5967.12214.
- Park S, Yoe SM (2017b). A novel cecropin-like peptide from black soldier fly, *Hermetia illucens*: Isolation, structural and functional characterization. Entomolog. Res. 47(2): 115–124. https://doi.org/10.1111/1748-5967.12226.
- Pinheiro J, Bates D, DebRoy S, Sarkar D, EISPACK, Heisterkamp S, Willigen B Van, R-core (2020). Linear and Nonlinear Mixed Effects Models.
- R Core Team (2020). R : A Language and Environment for Statistical Computing.
- Salavati ME, Rezaeipour V, Abdullahpour R, Mousavi N (2020). Effects of graded inclusion of bioactive peptides derived from sesame meal on the growth performance, internal organs, gut microbiota and intestinal morphology of broiler chickens. Int. J. Peptide Res. Therapeut. 26(3): 1541–1548. https://doi.org/10.1007/s10989-019-09947-8.
- Shang Y, Kumar S, Oakley B, Kim WK (2018). Chicken gut microbiota: Importance and detection technology. Front. Vet. Sci. 5: 1–11. https://doi.org/10.3389/fvets.2018.00254.
- St-Pierre NR (2001). Invited review: Integrating quantitative findings from multiple studies using mixed model methodology. J. Dairy Sci. 84(4): 741–755. https://doi.org/10.3168/jds.S0022-0302(01)74530-4.
- Tang X, Fatufe AA, Yin YL, Tang ZR, Wang SP, Liu ZQ (2012). Dietary supplementation with recombinant lactoferrampin-lactoferricin improves growth performance and affects serum parameters in piglets. J. Anim. Vet. Adv. 11: 2548–2555. https://doi.org/10.3923/javaa.2012.2548.2555.
- Torki M, Schokker D, Duijster-Lensing M, Van Krimpen MM (2018). Effect of nutritional interventions with quercetin, oat hulls, β-glucans, lysozyme and fish oil on performance and health status related parameters of broilers chickens. Brit. Poult. Sci. 59(5): 579–590. https://doi.org/10.1080/00071668.2018.1496402.
- Wallace P, Yang L (2010). Soybean peptide as additive on yellow feather broiler chicks: Nutritional and biochemical profiles. J. Ghana Sci. Assoc. 12(1): 59–67. https://doi.org/10.4314/jgsa.v12i1.56812.
- Wang D, Ma W, She R, Sun Q, Liu Y, Hu Y, Liu L, Yang Y, Peng K (2009). Effects of swine gut antimicrobial peptides on the intestinal mucosal immunity in specific-pathogen-free chickens. Poult. Sci. 88(5): 967–974. https://doi.org/10.3382/ps.2008-00533.

- Wang G, Song Q, Huang S, Wang Y, Cai S, Yu H, Ding X, Zeng X, Zhang J (2020). Effect of antimicrobial peptide microcin J25 on growth performance, immune regulation, and intestinal microbiota in broiler chickens challenged with *Escherichia coli* and *Salmonella*. Animals. 10(2): 345. https://doi.org/10.3390/ani10020345.
- Wang S, Zeng X, Yang Q, Qiao S (2016). Antimicrobial peptides as potential alternatives to antibiotics in food animal industry. Int. J. Molecul. Sci. 17(5): 603. https://doi.org/10.3390/ijms17050603.
- Wang S, Zeng XF, Wang QW, Zhu JL, Peng Q, Hou CL, Thacker P, Qiao SY (2015). The antimicrobial peptide sublancin ameliorates necrotic enteritis induced by *Clostridium perfringens* in broilers. J. Anim. Sci. 93(10): 4750–4760. https://doi.org/10.2527/jas.2015-9284.
- Wang Y, Shan T, Xu Z, Liu J, Feng J (2006). Effect of lactoferrin on the growth performance, intestinal morphology, and expression of PR-39 and protegrin-1 genes in weaned piglets. J. Anim. Sci. 84(10): 2636–2641. https://doi.org/10.2527/jas.2005-544.
- Wei L, Mu L, Wang Y, Bian H, Li J, Lu Y, Han Y, Liu T, Lv J, Feng C, Wu J, Yang H (2015). Purification and characterization of a novel defensin from the salivary glands of the black fly, *Simulium bannaense*. Parasit. Vectors. 8(71): 1–11. https://doi.org/10.1186/s13071-015-0669-9.
- Wen L-F, He J-G (2012). Dose–response effects of an antimicrobial peptide, a cecropin hybrid, on growth performance, nutrient utilisation, bacterial counts in the digesta and intestinal morphology in broilers. Brit. J. Nutrit. 108(10): 1756–1763. https://doi.org/10.1017/S0007114511007240.
- Xiao H, Shao F, Wu M, Ren W, Xiong X, Tan B, Yin Y (2015). The application of antimicrobial peptides as growth and health promoters for swine. J. Anim. Sci. Biotechnol. 6(19): 1–6. https://doi.org/10.1186/s40104-015-0018-z.
- Yeaman MR, Yount NY (2003). Mechanisms of antimicrobial peptide action and resistance. Pharmacol. Rev. 55(1): 27–55. https://doi.org/10.1124/pr.55.1.2.
- Yi H, Chowdhury M, Huang Y, Yu X-Q (2014). Insect antimicrobial peptides and their applications. Appl. Microbiol. Biotechnol. 98(13): 5807–5822. https://doi.org/10.1007/s00253-014-5792-6.
- Zhang G, Mathis GF, Hofacre CL, Yaghmaee P, Holley RA, Durance TD (2010). Effect of a radiant energy–treated lysozyme antimicrobial blend on the control of clostridial necrotic enteritis in broiler chickens. Avian Dis. Digest. 5(4): e43–e44. https://doi.org/10.1637/9549-937010-DIGEST.1.