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Antimicrobial peptides as an additive in broiler chicken nutrition: a meta-analysis of bird performance, nutrient digestibility and serum metabolites

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ABSTRACT. The present meta-analysis evaluated the effect of the level of antimicrobial peptides (AMPs) on broiler chicken growth performance, digestibility, small intestine morphology and blood serum parameters. The database was developed from 29 articles comprising 36 experiments. Data were analyzed using a mixed model methodology considering the levels of AMPs as fixed effects and different studies as random effects. It was shown that an increased AMPs addition level quadratically influenced body weight (BW), average daily gain (ADG) and feed conversion ratio (FCR) ($P < 0.05$). Simultaneously, it linearly reduced mortality ($P < 0.05$) both in the starter and finisher periods. There was a linear increase in metabolizable energy ($P < 0.05$). Small intestine morphology in the duodenum, as indicated by villus height and villus height to crypt depth ratio linearly increased, while the crypt depth was linearly decreased ($P < 0.05$). The mucosa thickness was quadratically affected in the jejunum, while the crypt depth linearly decreased ($P < 0.05$). Categorical analysis showed that AMPs had a comparable effect with antibiotics on broiler performance (BW, ADG, FCR) ($P > 0.05$); however the improvement in comparasion with the non-supplemented group was stated ($P < 0.01$). In conclusion, it is evidenced that AMPs can be used as an effective replacer of antibiotic growth promoters (AGP) because they can improve growth performance, digestibility, small intestine morphology and blood serum parameters of broiler in all rearing periods. Also, the optimal doses of dietary AMPs addition at 337 and 359 mg/kg of diet for the starter and finisher phases, respectively are suggested.

Introduction

Feed-added antibiotics have been widely used in the poultry industry because of their high ability to increase feed efficiency. However, such usage of antibiotics as growth promoters (AGP) may cause resistance and residue in broilers, and therefore many countries have banned the use of AGP (EFSA et al., 2019). Since then, searching for new AGP alternatives received substantial interest,

particularly in those that are originated or derived from nature such as antimicrobial peptides (AMPs) (Xiao et al., 2015; Wang et al., 2016). AMPs are a divergent group of small proteins that are characterized as strong cationic and heat-resistant. They have molecular weights ranging from 2.5 kDa to no more than 10 kDa with no residues and no adverse effect on eukaryotic cells (Xiao et al., 2015; Li et al., 2017).

AMPs have germicidal properties against Gram-positive and Gram-negative bacteria, fungi, viruses, phages and endoparasites (Li et al., 2017). Their mode of action has been well-explained in the literature, mainly regarding their ability to effectively disintegrate the microbe cell surface through the destruction of both cell membrane and nutrient transport system into the cell. Also, AMPs can interfere with the process of DNA transcription, RNA translation, protein synthesis and cell-level oxidation of pathogenic microbes (Xiao et al., 2015; Wang et al., 2016). AMPs provide an effective but not specific defence against infection (Wang et al., 2016). AMPs have been isolated from many natural sources such as mammals (e.g., defensin, colostrum and lactoferrin), amphibians (e.g., magainin), insects (e.g., cecropin and deptiricin), plants (e.g., thionin), microbes (e.g., gramicidin and nisin) and recombinant products (e.g., microcin J25, cecropinA (1-8)-magainin2 (1-12) and sarcotoxin IA) (Skosyrev et al., 2003; Xiao et al., 2015; Józefiak and Engberg, 2017). To date, more than 22,533 AMPs have been identified based on amino acid residues from various research databases (Zhao et al., 2013).

A growing number of studies have been conducted to assess the use of AMPs in broiler chickens nutrition whereas various degrees of effectiveness were found. The updated literature suggested that AMPs have a positive effect on the growth performance of broiler chickens both in the starter and finisher phases (Choi et al., 2013a,b). However, to date, there is no study attempting to quantitatively integrate empirical data regarding the use of AMPs in broiler chickens nutrition.

Meta-analysis is a statistical analysis aggregating results from scientific reports. It can produce a weighted average of the output, and the uncertainty value of the estimated equation can also be calculated (St-Pierre, 2001; Sauvant et al., 2008). A recent meta-analysis of antimicrobial additives has been reported by Vanrolleghem et al. (2019) and Xu et al. (2021) in swine feed. However, a meta-analysis of AMPs in broilers is not available. This study, therefore, aimed to (i) evaluate the effect of AMPs addition on growth performance, nutrient digestibility, small intestine morphology and blood serum parameters in broilers, and (ii) determine the optimal level of AMPs addition by employing a meta-analysis approach.

Material and methods

Publication searching strategy

Searching and collection of literature were carried out on the Internet databases as Google Scholar (https://scholar.google.com/) and Science Direct (https://www.sciencedirect.com/) by using various keywords such as 'antimicrobial peptide,' 'digestibility,' 'growth performance,' 'small intestine morphology,' 'blood serum,' and/or 'broiler'. There were 43 articles initially obtained using the previously mentioned keywords. To ensure the quality of the database, only articles from peer-reviewed journals were further assessed (Prihambodo et al., 2021). After strict evaluation of abstract and full texts, 29 articles comprising 36 experiments with a total of 111 data lines were used to develop the database as shown in Table 1.

In the database collected, the AMPs addition levels ranged between 0 (control) to 600 mg/kg of diet. The AMPs were derived from animal tissue purification (i.e. 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. canola, sesame and bioactive soyabean peptides). The broilers were reared in two phases, i.e. starter (1–21 days), finisher (22–42 days) and both periods. Broiler types involved in this meta-analysis varied: Arbor Acres, Cobb 500, Lingnan, Lohmann, Hubbard and ROSS 308.

The outcome variables included in the present study were: growth performance (e.g., body weight (BW), average daily gain (ADG), daily feed intake (DFI), feed conversion ratio (FCR) and mortality), nutrient digestibility, metabolism (e.g., dry matter digestibility (DMD), crude protein digestibility (CPD), apparent metabolizable energy (AME), crude fat digestibility (CFD)), small intestine morphology (e.g., mucosa thickness, villus height, crypt depth, villus height:crypt depth ratio (RVCD) in the duodenum, jejunum and ileum) and blood serum metabolites (e.g., total protein, albumin, globulin, albumin:globulin ratio (A:G ratio), cholesterol, triacylglycerol, creatinine and uric acid). The values of similar variables were converted into the same units to allow direct analysis within a particular variable. Only those variables with AMPs size greater than 5 ($n > 5$) were included in the analyses.

Table 1. Studies included in the meta-analysis

No	Study	Source of AMP	Type of AMP	Level	Strain	Sex	Period, days		
							starter	finisher	total
1	Jiang et al. (2009)	*Glycine max*	Soyabean bioactive peptides	0–200	Arbor Acres	NA	1–28	29–49	49
2	Wang et al. (2009)	Swine intestine	Swine antibacterial peptides	0–0.1	Lohmann	NA	–	–	42
3	Bao et al. (2009)	Swine intestine	Swine antibacterial peptides	0–200	Arbor Acres	Male	1–21	22–42	42
4	Ohh et al. (2009)	*Solanum tuberosum* L.	Refined potato protein	0–600	Ross 308	Male	1–21	22–42	42
5	Liu et al. (2010)	–	Lysozyme	0–40	Arbor Acres	Male	1–14	15–28	28
6	Han et al. (2010)	*Apis mellifera* L.	Bee venom	0–1	Arbor Acres	NA	1–28	–	28
7	Hu et al. (2010)	–	Glucagon-like peptide 2	0–0.33	Arbor Acres	NA	1–21	–	21
8	Zhang et al. (2010)	–	Lysozyme	0–200	Cobb 500	Male	1–28	–	28
9	Geier et al. (2011)	–	Bovine lactoferrin	0–500	Cobb 500	Male	1–24	25–32	32
10	Wen and He (2012)	*Hyalophora cecropia*	Cecropin AD-asparagin	0–8	Lingnan	Male	14–28	29–42	42
11	Choi et al. (2013a)	*Helicobacter pylori*	AMP-A3	0–90	Ross 308	NA	1–21	22–35	35
12	Choi et al. (2013b)	Analog of cecropin	AMP-P5	0–60	Ross 308	NA	1–21	22–35	35
13	Ali and Mohanny (2014)	*Apis mellifera carnica*	Bee venom	0–1.5	Ross 308	Mix	1–21	22–42	42
14	Aguirre et al. (2015)	–	Bovine lactoferrin	0–520	Cobb 500	NA	1–28	29–42	42
15	Wang et al. (2015)	*Bacillus subtilis*	Sublancin	0–11.52	Arbor Acres	NA	1–21	22–28	28
16	Karimzadeh et al. (2016)	*Brassica* spp.	Canola bioactive peptides	0–250	Ross 308	Male	1–28	29–42	42
17	Abdel-Latif et al. (2017)	–	Lysozyme	0–120	Ross 308	NA	1–21	22–35	35
18	Karimzadeh et al. (2017a)	–	Peptide	0–250	–	NA	1–28	29–42	42
19	Karimzadeh et al. (2017b)	*Brassica* spp.	Canola bioactive peptides	0–250	Ross 308	Male	1–28	29–42	42
20	Gong et al. (2017)	Egg white	Lysozyme	0–100	Ross 308	Male	1–24	25–35	35
21	Enany et al. (2017)	–	Lactoferrin	0–250	Hubbard	Mix	–	–	42
22	Kim et al. (2018)	*Apis mellifera*	Bee venom	0–0.5	Ross 308	Male	1–21	–	35
23	Torki et al. (2018)	Egg white	Lysozyme	0–40	Ross 308	Male	14–28	29–33	33
24	Ma et al. (2020)	*Saprophytic ascomycete*	Recombinant plectasin	0–200	Arbor Acres	Male	1–21	22–42	42
25	Daneshmand et al. (2020)	–	Camel lactoferrin chimera	0–20	Cobb 500	Male	1–10	11–24	24
26	Daneshmand et al. (2019)	–	Camel lactoferrin 36	0–20	Cobb 500	Male	1–22	–	22
27	Salavati et al. (2020)	*Sesamum indicum*	Sesame bioactive peptides	0–150	Ross 308	NA	1–24	25–35	35
28	Bai et al. (2019)	*Bombyx mori*	Cecropin	0–600	Arbor Acres	Mix	1–21	22–42	42
29	Wang et al. (2020)	–	Microcin J25	0–1	Arbor Acres	Male	1–21	22–42	42

AMP – antimicrobial peptide; NA – information is not available

Data analysis

Data analyses were conducted by statistical meta-analysis approach based on the mixed model methodology (St-Pierre, 2001; Sauvant et al., 2008). Experiments were served as random effects, while the AMPs addition level was considered as fixed effects. The statistical models used were as follow:

$$Y_{ij} = \beta_0 + \beta_1 \text{Level}_{ij} + \text{Experiment}_i + \text{Experiment}_i \text{Level}_{ij} + e_{ij} \quad (1),$$

$$Y_{ij} = \beta_0 + \beta_1 \text{Level}_{ij} + \beta_2 \text{Level}_{ij}^2 + \text{Experiment}_i + \text{Experiment}_i \text{Level}_{ij} + e_{ij} \quad (2)$$

where: (1) linear mixed model (LMM) mathematical model in the 1st order, (2) LMM mathematical model in the 2nd order, $\beta_0 + \beta_1 \text{Level}_{ij}$ (1st order) and $\beta_0 + \beta_1 \text{Level}_{ij} + \beta_2 \text{Level}_{ij}^2$ (2nd order) = fixed effect, $\text{Experiment}_i + \text{Experiment}_i \text{Level}_{ij}$ (1st and 2nd order), β_0 – overall intercept value across all experiments, β_1 – linear regression coefficient of 1st order, β_2 – linear regression coefficient of 2nd order, Level_{ij} – additional level on the random effect, Experiment – experiment_i, e_{ij} – unexplained residual errors.

The estimation of the coefficient followed the maximum likelihood method. The statistical models used were: *P*-values, root means square errors and Akaike information criterion (AIC). The results were declared to be significant at $P \leq 0.05$ and tended to be significant when the *P*-value was between 0.05 and 0.1. Initially, the model was assessed with the quadratic model and then altered to the linear model when the quadratic term was insignificant. For quadratic models, the optimum levels regarding AMPs concentration for the response variables were provided. We recognized that the minimum inhibitory concentration (MIC) and titer information are important factors. However, there is little available information on titer and MIC in poultry. Data were analyzed in the R software version 3.6.3 equipped with a 'nlme' library (Pinheiro et al., 2020; R Core Team, 2020).

The LMM models were used because the data is continuous. In this present meta-analysis, we did not perform an analysis based on the type of AMPs used because there has been a very limited number of data to implement a proper analysis for each AMPs. However, to examine the effectiveness of AMPs as AGP replacers, we categorized the database as discrete data into three groups as previously conducted by Irawan et al. (2020): (1) broiler chickens without commercial antibiotics – a control group; (2) broiler chickens receiving AGPs – an AGP group; and (3) broiler chickens receiving diet supplemented with AMPs – an AMP group. The discrete data in this meta-analysis were analyzed using the following model:

$$Y_{ij} = \mu + S_i + \tau_j + S\tau_{ij} + e_{ij},$$

where: Y_{ij} – predicted output for dependent variable Y, μ – overall mean, S_i – random effect of *i* study, τ_j – fixed effect of the *j* level, $S\tau_{ij}$ – random interaction between *i* study and the *j* level, and e_{ij} – unexplained residual error. A significance among groups was declared at $P < 0.05$ using least-square means and adjusted with a Tukey's test (Irawan et al., 2021).

Results

Regression equation of the effect of antimicrobial peptides doses on broiler chickens

In all phases, the broiler growth performance parameters such as BW, ADG and FCR improved significantly with the AMPs addition level ($P < 0.05$; Table 2). The AMPs addition effect on growth performance followed a quadratic pattern. In the starter phase, the AMPs levels to produce optimum BW, ADG and FCR were 337, 346 and 337 mg/kg of diet, respectively, with the predicted productivity for about 960 g (BW), 40.6 g/h/day (ADG) and 1.43 (FCR). To produce the optimum BW, ADG and FCR in the finisher phase, the AMPs levels were 352, 360 and 359 mg/kg of diet, respectively, with the predicted productivity for about 2260 g (BW), 76.6 g/h/day (ADG) and 1.96 (FCR). In the total phase, the addition of AMPs levels as much as 351, 412 and 371 mg/kg of diet resulted in the optimum BW, ADG and FCR, respectively. The predicted productivity was 1935 g (BW),

Table 2. The regression equation of the antimicrobial peptide (AMPs) addition (mg/kg of diet) on broiler growth performance in total phase

Response variables	Model	N	Variable estimates				Model estimates			Optimum output		
			int.	SE int.	slope	SE slope	*P*-value	RMSE	AIC	trend	X	Y
Growth performance in the starter phase												
BW, g	Q	82	912	43.6	0.29	0.085	0.001	1.82	942	Max.	337	960
	L				−0.000424	0.0002	0.007					
ADG, g/h/day	Q	82	38.5	1.90	0.0124	0.0043	0.006	1.99	446	Max.	346	40.6
	L				−1.80E−05	7.75E−06	0.025					
DFI, g/h/day	L	82	57.1	2.67	0.000392	0.0015	0.792	1.75	449	Pos.		
FCR	Q	82	1.52	0.04	−0.000546	0.0002	0.002	1.71	−118	Min	337	1.43
	L				1.00E−06	3.00E−07	0.01					
Growth performance in the finisher phase												
BW, g	Q	73	2.102	98.4	0.899	0.146	<0.001	1.64	927	Max.	352	2.260
	L				−0.00128	0.0003	<0.001					
ADG, g/h/day	Q	73	70.7	2.61	0.0327	0.006	<0.001	1.31	442	Max.	360	76.6
	L				−4.50E−05	1.08E−05	<0.001					
DFI, g/h/day	L	73	150	4.27	0.0027	0.0029	0.357	1.68	486	Pos.		
FCR	Q	73	2.15	0.067	−0.00106	0.0002	<0.001	1.83	−47.1	Min.	359	1.96
	L				1.00E−06	4.40E−07	0.001					
Growth performance evaluated in all phases												
BW, g	Q	101	1.752	115	1.04	0.145	<0.001	1.63	1.330	Max.	351	1.935
					−0.00148	0.0003	<0.001					
ADG, g/h/day	Q	106	54.4	4.46	0.0273	0.0039	<0.001	1.45	657	Max.	412	60
					−3.30E−05	7.37E−06	<0.001					
DFI, g/h/day	L	104	102	8.77	0.00279	0.0015	0.071	1.77	677	Pos.		
FCR	Q	104	1.9	0.0585	−0.000933	0.0002	<0.001	1.69	−107	Min.	371	1.73
					1.00E−06	2.90E−07	<0.001					
Mortality, %	L	17	12.2	4.56	−0.045	0.0157	0.017	1.08	123	Neg.		

ADG – average daily gain, AIC – Akaike information criterion, BW – body weight, DFI – daily feed intake, FCR – feed conversion ratio, int. – intercept, L – linear, Max. – maximum, Min. – minimum, N – number of data, Neg. – negative, Pos. – positive, Q – quadratic, RMSE – root mean square errors, SE – standard error, X – level (mg/kg of diet), Y – optimal value of response variables

60.0 g/h/day (ADG) and 1.73 (FCR). Also, in the total phase, the increased AMPs addition level significantly decreased mortality ($P < 0.05$) and tended to increase the DFI ($P < 0.10$). However, the increased AMPs addition level did not significantly increase the DFI in the starter and finisher phases.

Concerning the effect of AMPs on digestion and small intestine morphology, the digestibility of crude fat linearly increased ($P < 0.05$), but it affected quadratically the finisher phase ($P < 0.05$; Table 3). The AME significantly decreased in the finisher phase. The AMPs treatment did not affect DMD and CPD in the finisher phase. In the starter phase, levels of AMPs had no effect on AME and DMD; however, the tendency to decrease the CPD ($P < 0.10$) was observed. Several variables of small intestine morphology in the duodenum, such as villus height, crypt depth and RVCD, were significantly affected by the addition of AMPs ($P < 0.05$), except the mucosa thickness. In the jejunum, the mucosa thickness significantly increased ($P < 0.05$), while crypt depth tended to decrease ($P < 0.10$). Meanwhile, villus height and RVCD did not increase substantially. Also, there was no significant effect of AMPs addition on small intestine morphology in the ileum.

The effect of AMPs addition level on blood serum in broiler during starter, finisher, and total phases are presented in Table 4. In the starter period, the A:G ratio and triacylglycerol concentration were significantly decreased (linear; $P < 0.05$). In addition, globulin concentration tended to increase, while cholesterol concentration tended to decrease ($P < 0.10$). Total protein and albumin concentrations were not significantly affected by AMPs addition. In the finisher phase, the creatinine concentration changed quadratically due to the increase of AMPs inclusion ($P < 0.05$). Whereas total protein, albumin, globulin, cholesterol, triacylglycerol and uric acid concentrations and A:G ratio were not significantly affected by the increase of AMPs inclusion levels ($P > 0.10$).

Table 3. The regression equation of the antimicrobial peptides (AMPs) addition (mg/kg of diet) on digestibility and small intestine morphology of broiler

Response variables	Model	N	Variable estimates				Model estimates			Interpretation		
			int	SE int	slope	SE slope	*P*-value	RMSE	AIC	trend	X	Y
Digestibility and metabolizable energy in the starter phase												
dry matter, % FM	L	10	77.3	0.864	0.000782	0.0022	0.733	1.00	40.5	Pos.		
crude protein, % DM	L	19	66	3.71	−0.0075	0.0042	0.0989	1.11	114	Neg.		
crude fat, % DM	L	5	86.4	0.0616	0.495	0.0126	<0.001	1.00	−5.67	Pos.		
AME, kcal/kg	L	9	2.882	157	−0.209	0.375	0.598	0.94	126	Neg.		
Digestibility and metabolizable energy in the finisher phase												
dry matter, % FM	L	15	74.4	1.39	0.000749	0.0038	0.847	1.27	76.1	Pos.		
crude protein, % DM	L	20	68.1	1.17	−0.00208	0.0062	0.742	1.26	121	Neg.		
crude fat, % DM	Q	10	81.2	9.03	−0.131	0.0472	0.032	0.93	65	Min.	106	74.3
	L				0.00062	0.0002	0.0149					
AME, kcal/kg	L	5	2.985	14.8	32.4	3.02	0.0017	1.00	49.1	Pos.		
Small intestine morphology in the duodenum												
mucosa thickness, µm	L	6	708	52.2	0.271	0.506	0.63	1.02	77.6	Pos.		
villus height, µm	L	49	1048	120	0.562	0.188	0.0052	1.63	651	Pos.		
crypt depth, µm	L	43	229	49.5	−0.0921	0.0437	0.0436	1.90	454	Neg.		
RVCD	L	47	5.99	0.823	0.00472	0.0019	0.016	1.58	179	Pos.		
Small intestine morphology in the jejunum												
mucosa thickness, µm	Q	6	440	19.5	4.48	0.876	0.0362	0.97	64.2	Max.	102	668
	L				−0.022	0.0045	0.039					
villus height, µm	L	32	1138	324	1.23	3.93	0.757	2.40	566	Pos.		
crypt depth, µm	L	26	208	38.6	−0.126	0.0613	0.057	0.99	261	Neg.		
RVCD	L	30	6.87	2.1	0.000507	0.0249	0.984	2.30	225	Pos.		
Small intestine morphology in the ileum												
villus height, µm	L	30	678	138	0.304	0.209	0.16	1.64	394	Pos.		
crypt depth, µm	L	30	151	21	0.00962	0.0504	0.851	1.49	302	Pos.		
RVCD	L	34	4.6	0.773	0.00151	0.0025	0.547	1.73	135	Pos.		

AIC – Akaike information criterion, AME – apparent metabolizable energy, DM – dry matter, FM – fresh matter, Int. – intercept, L – linear, Max. – maximum, Min. – minimum, N – number of data, Neg. – negative, Pos. – positive, Q – quadratic, RMSE – root mean square errors, RVCD – ratio of villus height to crypt depth, SE – standard error, X – level (mg/kg of diet), Y – optimal value of response variables

Table 4. The regression equation of the antimicrobial peptides (AMPs) addition (mg/kg of diet) on blood serum of broilers

Response variables	Model	N	Variable estimates				Model estimates			Interpretation		
			int.	SE int.	slope	SE slope	*P*-value	RMSE	AIC	trend	X	Y
Blood serum in the starter phase												
total protein, g/dl	L	13	4.47	0.501	0.0018	0.0014	0.223	0.96	21.7	Pos.		
albumin, g/dl	L	8	3.23	0.258	−0.00189	0.0024	0.472	0.89	9.16	Neg.		
globulin, g/dl	L	8	1.68	0.66	0.00573	0.0025	0.072	0.94	12.7	Pos.		
A:G ratio	L	8	2.57	1.1	−0.0172	0.0022	<0.001	0.97	13.1	Neg.		
cholesterol, mg/dl	L	13	121	4.61	−0.0888	0.0396	0.052	1.30	106	Neg.		
triacylglycerol, mg/dl	L	9	90.1	40.1	−0.0639	0.0196	0.017	0.94	70.3	Neg.		
Blood serum in the finisher phase												
total protein, g/dl	L	18	17.8	13	−0.00414	0.0048	0.402	1.33	99.9	Neg.		
albumin, g/dl	L	13	6.13	4	9.90E−05	0.0033	0.977	1.14	50.1	Pos.		
globulin, g/dl	L	13	2.6	0.873	0.00272	0.0019	0.199	1.02	28.3	Pos.		
A:G ratio	L	13	1.75	0.622	−0.00265	0.0016	0.136	0.87	22	Neg.		
cholesterol, mg/dl	L	18	106	25.5	−0.0794	0.076	0.317	1.17	179	Neg.		
triacylglycerol, mg/dl	L	14	85.7	18.6	0.0232	0.0196	0.266	1.04	110	Pos.		
creatinine, mg/dl	Q	9	0.326	0.09	−0.000735	0.0002	0.031	1.00	−31.7	Min.	80.9	0.3
	L				5.00E−06	1.57E−6	0.045					
uric acid, mg/dl	L	9	6.68	0.416	−0.00382	0.0048	0.466	1.24	26.8	Neg.		

A:G ratio – albumin-globulin ratio, AIC – Akaike information criterion, int – intercept, L – linear, Min. – minimum; N – number of data, Neg. – negative, Pos. – positive, Q – quadratic, RMSE – root mean square errors, SE – standard error, X – level (mg/kg of diet), Y – optimal value of response variables

Table 5. Meta-analysis of the effect of antibiotics as growth promoters (AGPs) *vs* antimicrobial peptides (AMPs) on growth performance and small intestine morphology of broiler chickens

Response variables	AGPs *vs* AMPs			RMSE	*P*-value
	Control	AGPs	AMPs		
Growth performance in starter phases					
BW, g	905^{a}	1.069^{b}	895^{ab}	1.82	0.005
ADG, g/h/day	38.2^{a}	44.5^{b}	37.3^{a}	1.99	0.003
DFI, g/h/day	57.7^{a}	65.7^{b}	54.9^{a}	1.75	0.011
FCR	1.55^{b}	1.55^{ab}	1.50^{a}	1.71	<0.001
Growth performance in finisher phases					
BW, g	2.092^{a}	2.076^{ab}	2.096^{b}	1.64	<0.001
ADG, g/h/day	70^{a}	71.5^{b}	72.4^{b}	1.31	<0.001
DFI, g/h/day	150	146	148	1.68	0.99
FCR	2.18^{b}	2.07^{a}	2.05^{a}	1.83	<0.001
Growth performance in all phases					
BW, g	1.738^{a}	1.802^{b}	1.913^{b}	1.63	<0.001
ADG, g/h/day	49.7^{a}	53.9^{b}	57.2^{b}	1.45	<0.001
DFI, g/h/day	102	85.3	103	1.77	0.638
FCR	1.92^{b}	1.81^{a}	1.82^{a}	1.69	<0.001
mortality rate, %	16.6^{b}	9.40^{a}	5.07^{a}	1.08	0.001
Small intestine morphology in the duodenum					
mucosa thickness, µm	620^{a}	-	780^{b}	1.02	0.003
villus height, µm	1000^{a}	1107^{ab}	1425^{b}	1.63	<0.001
crypt depth, µm	234	345	212	1.90	0.074
RVCD	4.93^{a}	5.58^{ab}	6.3^{b}	1.58	0.003
Small intestine morphology in the jejunum					
villus height, µm	804	1138	1406	0.97	0.508
crypt depth, µm	210	282	209	2.40	0.09
RVCD	4.71	4.13	8.06	0.99	0.492
Small intestine morphology in the ileum					
villus height, µm	643	665	780	1.64	0.054
crypt depth, µm	159	210	143	1.49	0.386
RVCD	4.12	3.05	5.37	1.73	0.116

ADG – average daily gain, BW – body weight, DFI – daily feed intake, FCR – feed conversion ratio RMSE – root mean square errors, RVCD – ratio of villus height to crypt depth; ^{ab} – different superscript within the same row are significantly different at $P < 0.05$

Effect of antimicrobial peptides compared to antibiotics growth promoter

The comparison between AGP and AMPs groups of broiler chickens is presented in Table 5. In the starter period, broiler chickens that received AMPs had significantly lower BW and ADG in comparison to birds treated with AGP ($P < 0.01$). In finisher period and in the slaughter age, no difference was observed on BW and ADG when compared with the AGP group ($P > 0.05$) but they were higher than that of the control group in finisher period ($P < 0.01$). There was no difference in FCR between AMPs and AGP groups, but the AMPs group had significantly lower FCR when compared to the control group in all phases ($P < 0.01$). In comparison to the AGP and control groups, AMPs successfully improved mortality rate ($P < 0.01$). In addition, there was also no substantial change in all small intestinal sections between birds receiving AGP and AMPs ($P > 0.05$).

Discussion

Effect on growth performance of broiler chickens. In general, the addition of AMPs can improve broiler growth performance as indicated by the increase of BW and ADG, followed by the decrease of FCR and mortality, either in starter, finisher or total phases. A similar finding was proved by previous studies that used AMPs in the form of cecropin, AMP-A3 and AMP-P5 (Bai et al., 2019). The AMPs serves as an antimicrobial agent that could inhibit or even kill pathogenic microbes and improve the small intestine morphology so that digestion and nutrients absorption of broiler may be more optimal (Xiao et al., 2015; Józefiak and Engberg, 2017). In the study on another monogastric animal, such as pig, a similar response was shown (Yoon et al., 2013). This study also provided evidence that AMPs can be used as an AGP replacer as comparable effectivity to improve broiler chickens performance was noted. This is in agreement with recent studies using Microcin J25 and nisin (Kierończyk et al., 2020; Wang et al., 2020).

It was suggested that AMPs originated from different sources, either plant or animal, show a positive effect on broiler growth performance. For instance, the insect-derived AMPs, such as cecropin (*Hyalophora cecropia*) showed positive effects on DFI, and ADG, and reduced the FCR of the broiler chickens (Wen and He, 2012). The animal tissue-derived AMPs such as swine antibacterial peptide (200 mg/kg of diet) improved the broilers final body weight (Bao et al., 2009). In addition, plant-derived AMPs, such as canola meal bioactive peptide (200 mg/kg of diet), also positively increased ADG of broilers (Karimzadeh et al., 2017b).

Effect on digestibility and small intestine morphology of broiler chickens. The increase of CFD due to AMPs addition is an indirect effect of the improved small intestine morphology to optimize the nutrient absorption process (Feingold and Grunfeld, 2000). A tendency of the CPD decline by the addition of AMPs is highlighted in the present study. The decline of CPD was probably caused by the less specific AMPs action, since AMPs, mostly comprised of cationic charge, interacted with a negative charge of amino acids and formed chelating compounds (Selle et al., 2007). The decrease of CPD was also reported by Ohh et al. (2009) with added refined potato protein treatment. In contrast, Choi et al. (2013a) reported that the addition of AMP-A3 as much as 0–90 mg/kg linearly increased the CPD. The increase of AME was the indirect effect of AMPs addition due to improved health and small intestine morphology.

The positive effect of AMPs addition on small intestine morphology in the duodenum was supported by previous studies. Jin et al. (2008) reported that the addition of AMPs such as potato protein and lactoferrin showed a positive (linear) effect on villus height and RVCD, while the effect on crypt depth was negative (linear). Insect-derived AMPs, like cecropin, could inhibit pathogenic bacteria, such as *Escherichia coli*, coliform and the *Micrococcus luteus.* Thus, the inhibition effect increased the height of the villus and decreased crypt depth (Yi et al., 2014). Villus height was another factor, in addition to the number of villi that affected the area of villi in the small intestine. The increase of villus height in the duodenum had a beneficial effect on the contact of digestive enzymes with nutrients so that the nutrient degradation process and its distribution to the jejunum could be optimum (Svihus, 2014).

The positive effect of AMPs addition on small intestine morphology in the jejunum was slightly different according to Bao et al. (2009) who reported a significant increase of mucosa thickness and villus height with swine AMPs usage, and Wang et al. (2006) who observed that lactoferrin had significantly increased villus height and decreased crypt depth. In the jejunum, most fats, such as cholesterol, fatty acids and triacylglycerols are digested and then absorbed. The mucosa epithelium and villus serve an important role in this process (Svihus, 2014).

The present finding showed that AMPs did not affect the small intestine morphology (e.g., villus height, crypt depth and their ratio) in the ileum. Xiao et al. (2013), who used composite AMPs in swine diet, reported the opposite results. Although it was not significant, the AMPs addition had a positive effect on small intestine morphology in the ileum and ileum function to re-absorb bile salts and B_{12} vitamin (Svihus, 2014).

The AMPs served as an antimicrobial for pathogenic microbes through the damage of cell wall integrity and their intracellular activity (Xiao et al., 2015; Wang et al., 2016). Decreasing the pathogenic microbial population AMPs had a positive impact on beneficial bacteria such as lactic acid bacteria. Lactic acid bacteria in the digestive tract could improve the small intestine morphology by increasing the absorption area through the increased villus height. The number of villi could also decrease the crypt depth (Aliakbarpour et al., 2012). The improvement of small intestine morphology had a positive impact on the digestibility and metabolism of nutrients. However, there were still several variables that showed the decline pattern, such as the digestibility of crude protein and crude fat. This condition occurred because of the less specific AMPs action. Other nutrients (e.g., amino acids, fatty acids, vitamins and minerals) could be bound to form a complex compound (AMP-nutrients). A complex compound was more difficult to dissolve than a simple compound. Also, nutrient digestibility was influenced by various factors such as particle size, solubility, enzyme interaction, viscosity, temperature, acidity, digestive microbial composition and many other factors (Lesson and Summers, 2009).

Effect on blood serum of broiler chickens. It was stated that in the starter phase, the AMPs addition declined some blood serum variables such as total cholesterol, A:G ratio and triacylglycerol. A decrease in total cholesterol and triacylglycerol concentrations was also reported in the starter broiler phase due to the lysozyme addition at a dose of 90 mg/kg of diet. However, another study conducted on swine reported that the addition of zinc antibacterial peptide did not affect total cholesterol and triacylglycerol concnetrations (Abdel-Latif et al., 2017). Cholesterol and triacylglycerol are transported in the blood in the form of lipoproteins. The decrease of both components was likely due to their low proportion in lipoproteins since the high CFD in the starter phase was reported. Low-density lipoprotein, also called 'a bad fat,' is lipoprotein with a high cholesterol component (Bauer et al., 2005). The present finding was slightly different from the previous study that also displayed a significant effect on albumin and globulin concentrations and A:G ratio with a positive linear pattern (Xiao et al., 2015; Kim et al., 2018).

The addition of AMPs did not affect the entire blood serum of the finisher phase. However, there was a significant reduction of creatinine concentration with a quadratic pattern observed. The minimum creatinine (0.300 mg/dl) was noted when the AMPs level was about 80.93 mg/kg of diet. This finding was slightly higher in comparison to that of Kim et al. (2018) in which the range of creatinine concentration was about 0.210–0.239 mg/dl as the effect of AMPs addition in the form of bee venom at a dose of 0–0.5 mg/kg of diet in finisher broiler phase. Creatinine is mostly (95%) stored in muscle in the form of creatine phosphate, and then it is used as the main energy source during heavy work such as repairing damaged cells, increasing muscle mass and other working. The decrease of creatinine indicated a decline in creatinine use for those mentioned works. In opposite, the increased creatinine in blood serum indicates glomerular damage (Saks and Ventura-Clapier, 1994).

Furthermore, AMPs seem to have a positive effect on broiler blood serum metabolites in the starter phase and no effect in the finisher phase. This variation might correspond with the less specific action of AMPs or even other mechanisms. The significant reduction of creatinine concentration and a tendency to cholesterol concentration reduction as influenced by the increase of AMPs inclusion suggest that AMPs addition can improve the quality of livestock derived products.

Challenge and future direction. Despite sufficient and clear evidence of AMPs benefits on broiler chickens over conventional antibiotics, there are still challenges that need to be considered for the future direction. Nowadays, high production costs and the time consumed to produce AMPs in an industrial setting have become the drawbacks concerning that the demand for the use of AMPs is predicted to be continuously increased. To produce the AMPs from host-producing cells, it needs to kill the host (Tanhaiean et al., 2018; Tanhaieian et al., 2018). In particular, most of the AMPs reported in the studies compiled in the present meta-analysis were produced conventionally. Thus, the first take-home message is to develop and industrialize the most efficient production method. Regarding this, some studies can be acknowledged due to their successful strategy to produce and purify the AMPs

more efficiently by using a bioengineered approach (Tanhaeian et al., 2018; Tanhaieian et al., 2018 Tanhaeian et al., 2020).

Recombinant or synthetic AMPs of the chimeric peptide have been successfully expressed from a simple method by using *E. coli* (Tanhaeian et al., 2018) and *Lactococcus lactis* (Tanhaieian et al., 2018) with considerable antimicrobial and antioxidant activities. Other methods to optimize the stability and biocompatibility, reduce toxicity and prolong the site-specific retention of AMP polymer are developed with a nanostructure approach. It was reported that chimeric peptide analogues using the nanostructure technique were effective to inhibit some pathogens such as *Streptococcus mutans, Staphylococcus epidermidis* and *E. coli* (Tanhaeian et al., 2018; Tanhaieian et al., 2018). In addition, there is also little known regarding the specific mechanism on how AMPs work especially in the preclinical area such as pharmacokinetics, absorption, distribution, metabolism and excretion (Magana et al., 2020). Therefore, a future study in this area is to be investigated in animal livestock.

Conclusions

The present meta-analysis elucidates the positive effect of dietary antimicrobial peptides (AMPs) addition on broiler chickens growth performance, digestibility, small intestine morphology and blood serum parameters, which was observed on the starter and finisher phases. This study also recommends optimum AMPs dosage based on the feed conversion ratio either for starter or finisher periods at 337 and 359 mg/kg of diet, respectively.

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Conflict of interest

The authors declare that there is no conflict of interest.

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