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Antimicrobial peptides as additive: A meta-analysis on broiler chickens performance, nutrient digestibility, and serum metabolites

Type

Original paper

Keywords

antimicrobial peptide, broiler chickens, growth performance, meta-analysis

Abstract

The present meta-analysis evaluated the effect of level of antimicrobial peptides (AMPs) on growth performance, digestibility, small intestine morphology, and blood serum of broiler chickens. The database was developed from 29 published 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. The results showed an increased AMPs addition level quadratically influenced body weight, average daily gain, and feed conversion ratio ($P < 0.05$). Simultaneously, it linearly reduced mortality ($P < 0.05$) either in the starter and finisher periods. In the starter, there was a linear increased of crude fat digestion (CFD) and a linear decreased of triacylglycerol and the albumin to globulin ratio ($P < 0.05$). There were quadratic relationship between levels of AMPs with CFD and creatinine in the finisher, while it linearly increased 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 quadratically affected in the jejunum, while the crypt depth linearly decreased ($P < 0.05$). In conclusion, AMPs had positive effects to improve the growth performance, digestibility, small intestine morphology, and blood serum of broiler in all rearing periods. Also, this study suggested optimal doses of dietary AMPs addition at 337 and 359 mg kg−1 of diet for the starter and finisher phases, respectively.

Running title: Effect of antimicrobial peptides on broiler chickens

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Keywords: antimicrobial peptide; broiler chickens; growth performance; meta-analysis

Introduction

Feed-added antibiotics have been widely used in the poultry industry because of their high efficacy on increasing feed efficiency. However, such use of antibiotic as a growth promoters (AGP) may cause resistance and residue in broilers, and therefore many countries have banned the use of AGP (Bronzwaer et al., 2019). Since then, searching for new AGP alternatives received a substantial interest, particularly those originated or derived from mature such as antimicrobial peptides (AMPs) (Xiao et al., 2015; Wang et al., 2016). AMPs are oligopeptides compound that composed of 4 to 99 amino acids that characterized as strong cationic, heat-resistant (100°C for 15 min). They have molecular weights ranging from 2.5 kDa to more than 10 kDa with no residues and no adverse effect on eukaryotic cells (Xiao et al., 2015; Li et al., 2017).

The AMPs have germicidal properties against gram-positive, gram-negative bacteria, fungi, viruses, phages, and endoparasites (Li et al., 2017). Their mode of action have been well-explained in many literatures, 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 provides an effective but not specific defense against infection (Wang et al., 2016). AMPs have been isolated from many natural sources such as from 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 AMPs in broiler chickens whereas varied degrees of effectiveness were found. The updated literatures suggested that AMPs have a positive effect on growth performance of broiler chickens both in the starter and finisher phases (Choi et al., 2013). However, to date, there is no study attempting to quantitatively integrate empirical data regarding the use of AMPs on broiler chickens. In addition, it remains unclear how the process behind the improvement of growth performance and whether the inclusion levels of AMPs were too low or even inefficient. This study therefore aimed to (i) evaluate the effect of AMPs addition on growth performance, nutrient digestibility, small intestine morphology, and blood serum of broilers, and (ii) determine the optimal level of AMPs addition by employing meta-analysis approach.

Materials and Methods

Searching strategy of the literatures

Searching and collection of literature were carried out on 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 asure the quality of the database, further selection was conduted on the basis of abstract and fulltexts which results on 29 papers comprising 36 experiments with a total of 111 datalines, as shown in Table 1. The selection was made through the title, abstract, and fulltext evaluation by considering the presence of information dealing with AMPs

addition levels, growth performance, digestibility, small intestine morphology, and broiler blood serum.

In the database collected, the AMPs addition levels ranged between 0 (control) to 600 mg kg^{-1} of diet. The AMPs was 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 soybean 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 were varied, namely 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 per crypt depth ratio (RVCD) in the duodenum, jejunum, and ileum), and blood serum metabolites (e.g., total protein, albumin, globulin, albumin-globulin ratio [AGR], 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.

Data analysis

Data analyses were conducted by statistical meta-analysis approach based on 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:

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

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

Where (1) Linear mixed models (LMM) mathematical model in the 1st order, (2) LMM mathematical model in the 2nd order, $\beta_0 + \beta_1 Level_{ij}$ (1st order) and $\beta_0 + \beta_1 Level_{ij} + \beta_2 Level_{ij}^2$ (2nd order) = fixed effect, $Experiment_i + Experiment_i Level_{ij}$ (1st and 2nd order) = random effect, Y_{ij} = fixed variable, β_0 = overall intercept value across all experiments, β_1 = linear regression coefficient of 1st order, β_2 = linear regression coefficient of 2nd order, $Level_{ij}$ = the additional level on the random effect, Experiment = experiment -i, e_{ij} = the unexplained residual errors. The estimation of the coefficient followed the maximum likelihood method. The statistical models used were *p*-values, root mean square errors, and Akaike information criterion. The results were declared to be significant at $p \leq 0.05$ and tend 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. Data were analyzed in 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 type of AMPs used because there was very limited number of data in which not possible to implement a proper analysis for each AMPs. In addition, there is evidence that AMPs have a relatively similar mode of action regarding on their antibacterial activity (Zong et al., 2020).

Results

In all phases, the broiler growth performance 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 level to produce optimum BW, ADG, and FCR were 337, 346, and 337 mg kg^{-1} of diet, respectively, with the predicted productivity for about 960 g (BW), 40.6 g h^{-1} d^{-1} (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^{-1} of diet, respectively, with the predicted productivity for about 2260 g (BW), 76.6 g h^{-1} d^{-1} (ADG), and 1.96 (FCR). In the total phase, the addition of AMPs level as much as 351, 412, and 371 mg kg^{-1} of diet resulted in the optimum BW, ADG, and FCR, respectively. The predicted productivity was 1935 g (BW), 60.0 g h^{-1} d^{-1} (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 ($P < 0.1$). 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 in 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, it tended to decrease the CPD ($P < 0.1$). 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 were displayed in Table 4. In the starter broiler, the AGR and triacylglycerol were significantly decreased (linear; $P < 0.05$). In addition, globulin tended to increase, while cholesterol tended to decrease ($P < 0.1$). Total protein and albumin were not significantly affected by AMPs addition. In the finisher phase, the creatinine changedquadratically due to the increase of AMPs inclusion ($P < 0.05$). Whereas total protein, albumin, globulin, AGR, cholesterol, triacylglycerol, and uric acid were not significantly affected by the increase of AMPs inclusion levels ($P > 0.10$).

Discussion

Effect on growth performance of broiler chickens

In general, the addition of AMPs could 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 improved the small intestine morphology so that digestion and nutrients absorption of broiler was more optimal (Xiao et al., 2015; Józefiak and Engberg, 2017). Study in another monogastric animal, such as swine, showed a similar response (Yoon et al., 2013).

It was suggested that AMPs originated from a different source, either from plant or animal, shows 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 reduced the FCR of the broiler chickens (Wen et al., 2012). The animal tissue-derived AMPs such as swine antibacterial peptide (200 mg kg^{-1} of diet), improved the broiler's final bodyweight (Bao et al., 2009). In addition, plant-derived AMPs, such as canola meal bioactive peptide (200 mg kg^{-1} of diet), also positively increased ADG of broilers (Karimzadeh et al., 2017).

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). The present study also highlighted a tendency of the decline of CPD by the addition of AMPs. The decline of CPD was probably caused by the less specific of 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 crude protein digestibility was also reported by Ohh et al. (2009) with added refined potato protein treatment. In contrast, Choi et al. (2013) reported that the addition of AMP-A3 as much as 0–90 mg kg^{-1} 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 to 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 the villus that affected the area of villi in the

small intestine. The increase of villus height in the duodenum had a beneficial effect for the contact of digestive enzymes with nutrients so that the nutrient degradation process and its distribution to the jejunum could be optimum (Svihus et al., 2014).

The positive effect of AMPs addition on small intestine morphology in the jejenum was slightly different to (i) Bao et al. (2009)'s finding that used swine AMPs and reported a significant increase of mucosa thickness and villus height than control; and (ii) Wang et al. (2006)'s finding that lactoferrin had significantly increased villus height and decreased crypt depth. In the jejunum, most fats, such as cholesterol, fatty acids, and triacylglycerols, were digested and then absorbed. The mucosa epithelium and villus served 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 ratio) in the ileum. Xiao et al. (2013), who used composite AMPs in swine diet, reported the opposite results with the present finding. 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 B12 vitamins (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 had an impact on the increasing 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

In the starter phase, the AMPs addition declines some blood serum variables such as total cholesterol, RAG, and triacylglycerol. A decrease in total cholesterol and triacylglycerol was also reported in the starter broiler due to the lysozyme addition for about 90 mg kg^{-1} of diet. However, another study in swine reported that the addition of zinc antibacterial peptide did not affect total cholesterol and triacylglycerol (Abdel-Latif et al., 2017). Cholesterol and triacylglycerol were 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. Low-density lipoprotein, also called “a bad fat,” was lipoprotein with a high cholesterol component (Bauer et al., 2005). The present finding was slightly different from previous study that also displayed a significant effect on albumin and globulin and AGR with a positive linear pattern (Xiao et al., 2013; 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 with a quadratic pattern observed. The minimum creatinine (0.300 mg dL^{-1}) was noted in the AMPs level for about 80.93 mg kg^{-1} of diet. This finding was slightly higher compared to Kim et al. (2018) that reported the range of creatinine for about 0.210–0.239 mg dL^{-1} as the effect

of AMPs addition in the form of bee venom as much as 0–0.5 mg kg^{-1} of diet in finisher broiler. Creatinine was mostly (95%) stored in muscle in the form of creatine phosphate, and then it was 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 indicated glomerular damage (Dhalla, 1994).

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

Conclusion

The present meta-analysis elucidates the positive effect of dietary AMPs addition on broiler growth performance (i.e., BW, ADG, DFI, FCR and mortality), digestibility (i.e., AME and CFD), small intestine morphology (i.e., mucosa thickness, villus height, crypt depth and RVCD), and blood serum (i.e., globulin, cholesterol and creatinine) that were observed on starter and finisher phase. This study also recommends optimum AMPs dosage based on the FCR either for starter or finisher periods at 337 and 359 mg kg^{-1} of diet, respectively.

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Declaration of Interest Statement

Authors have declared that no competing interests exist.

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Table 1. Studies included in the meta-analysis

No	Study	Source of AMP	Type of AMP	Level	Strain	Sex	Period (d)		
							Starter	Finisher	Total
1	Jiang *et al.* 2009	*Glycine max*	Soybean 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 et al. 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.* 2019	*Saprophytic ascomycete*	Recombinant plectasin	0-200	Arbor Acres	Male	1-21	22-42	42
25	Daneshmand *et al.* 2019a	-	Camel lactoferrin chimera	0-20	Cobb 500	Male	1-10	11-24	24

488	26	Daneshmand *et al.* 2019b	-	Camel lactoferrin 36	0-20	Cobb 500	Male	1-22	-	22
489	27	Salavati *et al.* 2019	*Sesamum indicum*	Sesame bioactive peptides	0-150	Ross 308	NA	1-24	25-35	35
490	28	Bai *et al.* 2019	*Bombyx mori*	Cecropin	0-600	Arbor Acres	Mix	1-21	22-42	42
491	29	Wang *et al.* 2020	-	Microcin J25	0-1	Arbor Acres	Male	1-21	22-42	42

492 Note: AMP, antimicrobial peptide; NA, information is not available

Table 2. The regression equation of the AMPs addition (mg kg^{-1} of diet) on growth performance of broiler Growth performance in total phase

Response variables	Unit	Model	N	Variable estimates				Model estimates			Optimum output		
				Int.	SE Int.	Slope	SE Slope	p-value	RMSE	AIC[1]	Trend	X^2	Y^3
Growth performance in starter phase													
BW	g	Q	82	912	43.6	0.29	0.085	0.001	1.82	942	Max.	337	960
						-0.000424	0.0002	0.007					
ADG	g h^{-1} d^{-1}	Q	82	38.5	1.90	0.0124	0.0043	0.006	1.99	446	Max.	346	40.6
						-1.80E-05	7.75E-06	0.025					
DFI	g h^{-1} d^{-1}	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
						1.00E-06	3.00E-07	0.01					
Growth performance in finisher phase													
BW	g	Q	73	2,102	98.4	0.899	0.146	<0.001	1.64	927	Max.	352	2,260
						-0.00128	0.0003	<0.001					
ADG	g h^{-1} d^{-1}	Q	73	70.7	2.61	0.0327	0.006	<0.001	1.31	442	Max.	360	76.6
						-4.50E-05	1.08E-05	<0.001					
DFI	g h^{-1} d^{-1}	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
						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^{-1} d^{-1}	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^{-1} d^{-1}	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.		

Note : ADG = average daily gain; AIC = Akaike information criterion; DFI = daily feed intake; FCR = feed conversion ratio; Int = Intercept; L = linear; Max = maximum; Min = minimum; N = number of data; Neg = Negative; Q = quadratic; RMSE = root mean square errors; SE = standard error; AIC[1] = an estimator of the relative quality of statistical models for a given set of data; X^2 = level (mg kg^{-1} of diet); Y^3 = optimal value of response variables.

Table 3. The regression equation of the AMPs addition (mg kg^{-1} of diet) on digestibility and small intestine morphology of broiler

Response variables	Unit	Model	N	Variable estimates				Model estimates			Interpretation		
				Int.	SE Int.	Slope	SE Slope	p-value	RMSE	AIC[1]	Trend	X[2]	Y[3]
Digestibility and metabolizable energy in starter phase													
Dry matter	%	L	10	77.3	0.864	0.000782	0.0022	0.733	1.00	40.5	Pos.		
Crude protein	%	L	19	66	3.71	-0.0075	0.0042	0.0989	1.11	114	Neg.		
Crude fat	%	L	5	86.4	0.0616	0.495	0.0126	<0.001	1.00	-5.67	Pos.		
AME	kcal kg^{-1}	L	9	2,882	157	-0.209	0.375	0.598	0.94	126	Neg.		
Digestibility and metabolizable energy in finisher phase													
Dry matter	%	L	15	74.4	1.39	0.000749	0.0038	0.847	1.27	76.1	Pos.		
Crude protein	%	L	20	68.1	1.17	-0.00208	0.0062	0.742	1.26	121	Neg.		
Crude fat	%	Q	10	81.2	9.03	-0.131	0.0472	0.032	0.93	65	Min.	106	74.3
						0.00062	0.0002	0.0149					
AME	kcal kg^{-1}	L	5	2,985	14.8	32.4	3.02	0.0017	1.00	49.1	Pos.		
Small intestine morphology in 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	1,048	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.		

Response variables	Unit	Model	N	Variable estimates				Model estimates			Interpretation		
				Int.	SE Int.	Slope	SE Slope	p-value	RMSE	AIC[1]	Trend	X[2]	Y[3]
Small intestine morphology in jejunum													
Mucosa thickness	µm	Q	6	440	19.5	4.48	0.876	0.0362	0.97	64.2	Max.	102	668
						-0.022	0.0045	0.039					
Villus height	µm	L	32	1,138	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 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.		

Note : AIC = akaike information criterion; AME = apparent metabolizable energy; Int = intercept; L = linear; Max = maximum; Min = minimum; N = number of data; Neg = Negative; Q = quadratic; Pos = positive; RMSE = root mean square errors; RVCD = ratio of villus height to crypt depth; SE = standard error; AIC[1] = an estimator of the relative quality of statistical models for a given set of data; X[2] = level (mg kg^{-1} of diet); Y[3] = optimal value of response variables.

Table 4. The regression equation of the AMPs addition (mg kg^{-1} of diet) on blood serum of broilers

Response variables	Unit	Model	N	Variable estimates				Model estimates			Interpretation		
				Int.	SE Int.	Slope	SE Slope	p-value	RMSE	AIC[1]	Trend	X[2]	Y[3]
Blood serum in starter phase													
Total protein	g dL^{-1}	L	13	4.47	0.501	0.0018	0.0014	0.223	0.96	21.7	Pos.		
Albumin	g dL^{-1}	L	8	3.23	0.258	-0.00189	0.0024	0.472	0.89	9.16	Neg.		
Globulin	g dL^{-1}	L	8	1.68	0.66	0.00573	0.0025	0.072	0.94	12.7	Pos.		
AGR		L	8	2.57	1.1	-0.0172	0.0022	<0.001	0.97	13.1	Neg.		
Cholesterol	mg dL^{-1}	L	13	121	4.61	-0.0888	0.0396	0.052	1.30	106	Neg.		
Triacylglycerol	mg dL^{-1}	L	9	90.1	40.1	-0.0639	0.0196	0.017	0.94	70.3	Neg.		
Blood serum in finisher phase													
Total protein	g dL^{-1}	L	18	17.8	13	-0.00414	0.0048	0.402	1.33	99.9	Neg.		
Albumin	g dL^{-1}	L	13	6.13	4	9.90E-05	0.0033	0.977	1.14	50.1	Pos.		
Globulin	g dL^{-1}	L	13	2.6	0.873	0.00272	0.0019	0.199	1.02	28.3	Pos.		
AGR		L	13	1.75	0.622	-0.00265	0.0016	0.136	0.87	22	Neg.		
Cholesterol	mg dL^{-1}	L	18	106	25.5	-0.0794	0.076	0.317	1.17	179	Neg.		
Triacylglycerol	mg dL^{-1}	L	14	85.7	18.6	0.0232	0.0196	0.266	1.04	110	Pos.		
Creatinine	mg dL^{-1}	Q	9	0.326	0.09	-0.000735	0.0002	0.031	1.00	-31.7	Min.	80.9	0.3
						5.00E-06	1.57E-6	0.045					
Uric acid	mg dL^{-1}	L	9	6.68	0.416	-0.00382	0.0048	0.466	1.24	26.8	Neg.		

Note : AGR = albumin-globulin ratio; AIC = akaike information criterion; 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; AIC[1] = an estimator of the relative quality of statistical models for a given set of data; X[2] = level (mg kg^{-1} of diet); Y[3] = optimal value of response variables.

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