




# The effect of plectasin supplementation on small intestines morphometric characteristics, blood profile, and growth performance of broiler chickens

Avaliação da suplementação de plectasina nas características morfométricas do intestino delgado, perfil sanguíneo e desempenho de frangos de corte

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**Abstract:** Antimicrobial peptides such as plectasin have been proposed as a suitable replacement for antibiotic growth promoters in livestock. However, its efficacy under local conditions in philippines has yet to be determined. This study was conducted to evaluate plectasin's efficacy on growth performance, morphometric features of the small intestines, and selected blood parameters in broiler chickens. Three-hundred, 1-day-old Ross broiler chicks were randomly allocated using a randomized complete block design with initial weight as a blocking factor to the following treatment groups: negative control (T1); 250 ppm enramycin or positive control (T2); 150 ppm plectasin (T3); 300 ppm plectasin (T4); and 450 ppm plectasin (T5). Plectasin supplementation at 150 ppm during the finisher phase improved the growth performance of broiler chickens ( $P < 0.05$ ) by enhancing the feed conversion ratio  $1.89 \pm 0.12$  and increasing the average daily gain ( $80.68 \pm 5.40$ g). All treatment groups' average daily feed intake was comparable throughout the feeding phases. Although, supplementation did not affect morphometric measurements of the small intestines and serum levels of glucose, triglycerides, and cholesterol. This study indicates that the antimicrobial peptide plectasin has beneficial effects on growth performance and improve nutrient utilization efficiency without disrupting normal physiological functions.

**Keywords:** plectasin; blood profile; growth performance; intestinal morphology; broilers.

**Resumo:** Peptídeos antimicrobianos, como a plectasina, foram propostos como substitutos adequados para antibióticos promotores de crescimento em animais de produção. Entretanto, sua eficácia nas condições locais das Filipinas ainda não foi determinada. Este estudo foi conduzido para avaliar a eficácia da plectasina no desempenho do crescimento, nas características morfométricas do intestino delgado e em parâmetros sanguíneos de frangos de corte. Trezentos frangos de corte da linhagem Ross de um dia de idade foram alocados aleatoriamente utilizando um delineamento em blocos completos casualizados, tendo o peso inicial como fator de bloqueio, nos seguintes grupos de tratamento: controle negativo (T1); 250 ppm de enramicina ou controle positivo (T2); 150 ppm de plectasina (T3); 300 ppm de plectasina (T4); e 450 ppm de plectasina (T5). A suplementação de plectasina a 150 ppm durante a fase de terminação melhorou o desempenho de crescimento dos frangos de corte ( $P < 0,05$ ), aumentando a taxa de conversão alimentar em  $1,89 \pm 0,12$  e aumentando o ganho médio diário ( $80,68 \pm 5,40$ g). O consumo médio diário de ração de todos os grupos de tratamento foi comparável durante todas as fases de alimentação. No entanto,





a suplementação não afetou as medidas morfométricas do intestino delgado e os níveis séricos de glicose, triglicerídeos e colesterol. Esse estudo indica que o peptídeo antimicrobiano plectasina tem efeitos benéficos sobre o desempenho do crescimento e melhora a eficiência da utilização de nutrientes sem interromper as funções fisiológicas normais.

**Palavras-chave:** plectasina; perfil sanguíneo; desempenho; morfologia intestinal; frangos de corte.

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## 1. Introduction

Globally, the poultry industry has shown significant growth over the past few decades and remains one of the most dynamic and rapidly expanding sectors in animal agriculture. Considered the most progressive animal enterprise today <sup>(1)</sup>, the Philippine poultry industry boasted a 78 % growth from 1990 to 2016, valued at Philippines Peso (PhP) 69 billion in 1990 to PhP 123 billion in 2016. Poultry production in the Philippines grew by 6.99 % in the last quarter of 2018, contributing 16.18 % to total agricultural output. However, in 2020, the gross value of chicken production declined, falling by 8.5 % at current prices and 6.1 % at constant prices compared to 2019 <sup>(2)</sup>.

For the industry to continue to flourish and meet the needs of the increasing population, the maintenance of a healthy flock cannot be overemphasized. To achieve this, antibiotics for growth promotion have been extensively used in farms around the world <sup>(3)</sup>. However, the continuous use of antibiotics has given rise to the emergence of bacterial antibiotic resistance <sup>(4)</sup>. Antimicrobial resistance (AMR) in a broad range of infectious agents is a growing public and animal health concern <sup>(5)</sup> and this has prompted research for possible alternatives to antibiotics that can uphold the advantages it provides in terms of health and economics that have changed intensive poultry and livestock rearing <sup>(6)</sup>.

Antimicrobial peptides (AMP) are a prospective alternative to antibiotics in this situation for it has a broad spectrum of activity against bacteria, minimal risk of inducing bacterial resistance, and improve the growth performance <sup>(7)</sup>. Plectasin is a defensin-type AMP derived from the fungus *Pseudoplectania nigrella* <sup>(8)</sup>. Plectasin works by binding to the bacterial cell wall precursor lipid II, disrupting cell wall synthesis, which is essential for bacterial survival. Its called immunomodulatory effects that helping to maintain intestinal barrier function and support beneficial microbiota <sup>(9)</sup>.

Published studies have confirmed the possible efficacy of plectasin and plectasin-derived compounds in battling multi-drug-resistant Gram-positive bacteria like methicillin-resistant *Staphylococcus aureus* <sup>(7,10)</sup> and have a beneficial effect on growth performance of yellow feathered chickens at 21 days age <sup>(11)</sup>. The lack of studies on plectasin use under in the Philippine poultry sector, the emergence of AMR, and the restrictions imposed by government agencies on the use of in-feed antibiotics have driven the need to conduct this study. This research aims to determine the effects of plectasin on growth performance, small intestine morphometry, and selected blood serum parameters of broiler chickens.

## 2. Material and methods

All procedures performed in broiler chickens were approved by the Institutional Animal Care and Use Committee (IACUC) of the College of Veterinary Medicine, University of the Philippines Los Baños (UPLB) No 2018-0043.

### 2.1 Birds, housing, and management

Three hundred unsexed day-old Ross 308 strain broiler chicks were weighed (initial body weight =  $37 \pm 2$  g) randomly allocated to each treatment group (60 birds per treatment divided into 6 replication) and subjected to similar environmental conditions and management practices. The birds were housed



in raised single-tiered battery cages with wire flooring in the poultry house of the University of the Philippines Los Baños (UPLB) Veterinary Teaching Hospital (VTH) – Maahas Station. The cages have a dimension of 119.00 cm in length, 87.99 cm in width, 57.99 cm in height, and an elevation of 54.99 cm. The birds were raised according to standard management practices for broiler production. The birds were fed a starter diet from days 1 – 10, a grower diet from days 11 – 24, and a finisher diet from days 25 – 35 in crumble form (Ross Broiler Nutrition/Aviagen). Booster vaccination against Newcastle disease (HIPRAVIAR CLON CL/79, Hipra, Spain) was done on day 10 and day 18 via the ocular and nasal routes. Feed and water were given *ad libitum*. Environmental temperature and humidity were recorded daily.

## 2.2 Research design

The study utilized the randomized complete block design with initial weight as the blocking factor. There were five <sup>(5)</sup> treatment groups in the experiment: basal diet or negative control (T1); basal diet + 250 ppm enramycin or positive control (T2); basal diet + 150 ppm plectasin (T3); basal diet + 300 ppm plectasin (T4); and basal diet + 450 ppm plectasin (T5). The proximate analysis of feeds used in the starter, grower, and finisher phases was shown in Table 1.

**Table 1.** Nutrient composition of the feed used in the experiment.

Items	Treatments				
	negative control	250 ppm enramycin (positive control)	150 ppm plectasin	300 ppm plectasin	450 ppm plectasin
Starter Phase					
% M	10.22 ± 0.59	10.19 ± 0.63	9.97 ± 0.74	10.88 ± 0.11	9.85 ± 0.14
% Ash	5.48 ± 0.27	5.45 ± 0.13	5.66 ± 0.36	5.50 ± 0.42	5.32 ± 0.04
% CP	20.60 ± 0.70	20.00 ± 0.57	20.54 ± 0.05	21.53 ± 0.45	20.34 ± 0.49
% Cfa	6.19 ± 0.43	5.75 ± 0.23	6.36 ± 0.25	6.39 ± 0.63	6.26 ± 0.34
% Cfi	3.36 ± 0.31	3.63 ± 0.47	3.30 ± 0.21	3.22 ± 0.30	3.27 ± 0.13
Grower Phase					
% M	10.57 ± 0.47	9.84 ± 0.33	9.23 ± 0.43	9.45 ± 0.09	9.79 ± 0.34
% Ash	5.36 ± 0.22	5.30 ± 0.04	5.47 ± 0.23	5.30 ± 0.19	5.30 ± 0.12
% CP	20.37 ± 0.10	20.23 ± 0.38	20.21 ± 0.15	20.27 ± 0.59	20.32 ± 0.20
% Cfa	7.59 ± 0.16	6.91 ± 0.24	7.33 ± 0.15	7.21 ± 0.14	7.70 ± 0.26
% Cfi	3.26 ± 0.10	3.26 ± 0.01	3.42 ± 0.14	2.80 ± 0.34	2.65 ± 0.11
Finisher Phase					
% M	10.38 ± 0.14	10.09 ± 0.04	10.21 ± 0.24	10.26 ± 0.17	10.08 ± 0.05
% Ash	5.40 ± 0.23	5.69 ± 0.06	5.63 ± 0.20	5.44 ± 0.23	4.93 ± 0.20
% CP	19.79 ± 0.51	18.63 ± 0.35	19.25 ± 0.44	19.69 ± 0.21	18.27 ± 0.11
% Cfa	7.53 ± 0.35	7.55 ± 0.32	7.41 ± 0.33	7.72 ± 0.22	7.78 ± 0.42
% Cfi	3.41 ± 0.14	3.28 ± 0.19	3.49 ± 0.09	3.43 ± 0.12	3.68 ± 0.18

M = moisture; CP = crude protein; Cfi = crude fiber; Cfa = crude fat.

The feeds used were sourced from a local feed mill. For the startet mash, plectasin (Fun-Tide<sup>®</sup>, Guangdong Hinabiotech Co. Ltd, China) and enramycin (Enfrocin<sup>®</sup>, China Jiangsu Jintan Orientech Co. Ltd., China) were top dressed on the basal diet. Proximate analysis of the basal diet for each feeding phase was done to evaluate the nutritional content of the feeds. Moisture and ash content were determined in



duplicate according to the Association of Official Analytical Chemists procedure (AOAC). Crude protein content was assessed by the standard Kjeldahl copper catalyst method as reported in AOAC. Crude fat was determined using the Soxhlet method <sup>(12)</sup>.

## 2.3 Sample collection and measurements

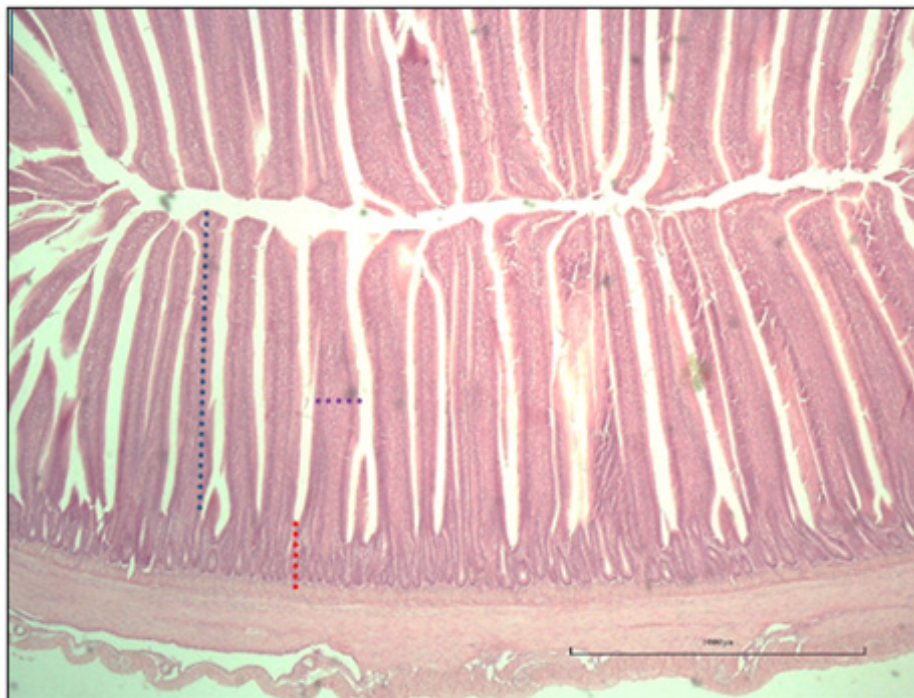
### 2.3.1 Growth production parameters

The birds were monitored for their daily feed consumption for the duration of the experiment. Body weight per replicate was measured on days 11, 25, and 35. Using the values obtained for body weight and feed consumption, average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR) were calculated for each treatment group.

### 2.3.2 Small intestine morphometry

A total of 60 birds (two birds per replicate) were sacrificed using atlanto-occipital dislocation on day 35. Intestinal segments from the duodenum (1.27 cm) were collected from the duodenal loop; segments from the jejunum (2.54 cm) were collected proximal to the Meckel's diverticulum; and segments from the ileum (3.81 cm) were collected proximal to the ileocecal junction. Samples were fixed in 10 % formalin, routinely processed for paraffin technique, sectioned, and stained with Hematoxylin and Eosin.

Tissue slides were examined under light microscopy (Nikon® Eclipse E200, Japan). A total of 30 properly oriented villi for each treatment were measured for villus width, villus height, and crypt depth in  $\mu\text{m}$  (Fig. 1). Measurements obtained from these parameters were used in the calculation of villus height: villus volume, crypt depth ratio, and villus surface area. The formulas for these measurements were derived from the studies of Balan <sup>(13)</sup>. Villus height was defined as the length between the crypt: villus transition zone and the villus apex while crypt depth was defined as the length between the base and the crypt: villus transition zone <sup>(14)</sup>. Villus surface area (VSA) was calculated using the formula:  $VSA = 2\pi (\text{villus width}/2) \times \text{villus height}$ .



**Figure 1.** Villus micrograph showing the methods of measuring the histomorphometric parameters. Blue line indicates villus height; red line indicates crypt depth; and purple line indicates villus width. H & E. Scale bar = 1000  $\mu\text{m}$ .



## 2.4 Blood parameters

Blood samples from two birds per replicate in each treatment were collected from the wing vein on day 35 and immediately analyzed for glucose, triglyceride, and cholesterol levels using an automated blood chemistry analyzer (Spotchem EZ, Japan).

## 2.5 Statistical analysis

Data were tested for normality, homogeneity of variances, and independence of errors using the Shapiro–Wilk test, Bartlett’s test, and Runs test, respectively. For variables meeting the assumptions of parametric analysis, treatment means were compared using one-way analysis of variance (ANOVA) under a Completely Randomized Design (CRD), followed by Tukey’s Honest Significant Difference (HSD) test for pairwise comparisons. For nonparametric data, the Kruskal–Wallis test was used, followed by the Dwass–Steel–Critchlow–Fligner (DSCF) method for multiple comparisons.

For variables analyzed with consideration of blocks, a Randomized Complete Block Design (RCBD) was used. Parametric data in RCBD were analyzed with ANOVA followed by Tukey’s HSD, while nonparametric data were analyzed using Friedman’s test, followed by Least Squares Rank (LSR) analysis. The level of statistical significance was set at  $P < 0.05$ . All analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA).

## 3. Results and discussion

The intestinal morphometric evaluation of the duodenum showed significantly higher villus height in negative control group whereas a shorter villus height was observed in 150 ppm plectasin group. However, the significantly shorter crypt depth in 150 ppm plectasin group, resulted in a high villus height to crypt depth ratio in this group. Other parameters such as villus width, villus volume, and villus surface area were comparable among treatment groups (Table 2).

In the jejunum, positive control (250 ppm enramycin) had a significantly shorter villus height and higher crypt depth that resulted in a lower villus height to crypt depth ratio, villus volume, and villus surface area. The morphometric measurements in the groups were comparable with each other (Table 2).

In the ileum, significantly lower villus width, crypt depth, villus volume, and surface area were observed in negative control group whereas 450 ppm plectasin group had a comparatively higher villus height and shallow crypt depth that resulted in a higher villus height to crypt depth ratio, villus volume, and villus surface area. Villus width and villus surface area were highest in 300 ppm plectasin group but villus height and villus height to crypt depth ratio was lowest in this group. Additionally, high crypt depth was seen in 150 ppm plectasin (Table 2).



**Table 2.** Mean ( $\pm$ SD) morphometric measurements of the small intestine of broiler chickens supplemented with plectasin.

Treatment Groups					
	negative control	250 ppm enramycin (positive control)	150 ppm plectasin	300 ppm plectasin	450 ppm plectasin
<b>Duodenum</b>					
VH (mm)	1.52 $\pm$ 0.16 <sup>a</sup>	1.38 $\pm$ 0.21 <sup>cd</sup>	1.30 $\pm$ 0.28 <sup>d</sup>	1.40 $\pm$ 0.27 <sup>bc</sup>	1.48 $\pm$ 0.20 <sup>ab</sup>
VW (mm)	0.19 $\pm$ 0.06	0.21 $\pm$ 0.08	0.19 $\pm$ 0.06	0.20 $\pm$ 0.06	0.21 $\pm$ 0.06
CD (mm)	0.30 $\pm$ 0.08 <sup>ab</sup>	0.28 $\pm$ 0.08 <sup>b</sup>	0.23 $\pm$ 0.05 <sup>c</sup>	0.33 $\pm$ 0.11 <sup>a</sup>	0.30 $\pm$ 0.10 <sup>ab</sup>
VH:CD	5.63 $\pm$ 2.20 <sup>a</sup>	5.35 $\pm$ 2.06 <sup>ab</sup>	5.76 $\pm$ 1.45 <sup>a</sup>	4.65 $\pm$ 1.57 <sup>b</sup>	5.52 $\pm$ 1.73 <sup>ab</sup>
VV (mm <sup>3</sup> )	0.0014 $\pm$ 0.0005	0.0015 $\pm$ 0.0007	0.0013 $\pm$ 0.0005	0.0014 $\pm$ 0.0005	0.0015 $\pm$ 0.0006
VSA(mm <sup>2</sup> )	0.93 $\pm$ 0.31	0.98 $\pm$ 0.49	0.84 $\pm$ 0.36	0.92 $\pm$ 0.31	1.00 $\pm$ 0.39
<b>Jejunum</b>					
VH (mm)	0.95 $\pm$ 0.22 <sup>a</sup>	0.80 $\pm$ 0.19 <sup>b</sup>	0.95 $\pm$ 0.25 <sup>a</sup>	0.93 $\pm$ 0.19 <sup>a</sup>	0.89 $\pm$ 0.15 <sup>a</sup>
VW (mm)	0.13 $\pm$ 0.05 <sup>a</sup>	0.12 $\pm$ 0.07 <sup>a</sup>	0.12 $\pm$ 0.03 <sup>a</sup>	0.12 $\pm$ 0.04 <sup>a</sup>	0.14 $\pm$ 0.04 <sup>a</sup>
CD (mm)	0.19 $\pm$ 0.05 <sup>c</sup>	0.24 $\pm$ 0.07 <sup>a</sup>	0.20 $\pm$ 0.07 <sup>bc</sup>	0.23 $\pm$ 0.06 <sup>a</sup>	0.22 $\pm$ 0.06 <sup>ab</sup>
VH:CD	5.22 $\pm$ 1.82 <sup>a</sup>	3.74 $\pm$ 1.75 <sup>c</sup>	5.00 $\pm$ 1.63 <sup>ab</sup>	4.26 $\pm$ 1.36 <sup>bc</sup>	4.35 $\pm$ 1.75 <sup>bc</sup>
VV (mm <sup>3</sup> )	0.0004 $\pm$ 0.0002 <sup>a</sup>	0.0003 $\pm$ 0.0002 <sup>b</sup>	0.0004 $\pm$ 0.0002 <sup>ab</sup>	0.0003 $\pm$ 0.0001 <sup>ab</sup>	0.0004 $\pm$ 0.0001 <sup>a</sup>
VSA(mm <sup>2</sup> )	0.42 $\pm$ 0.20 <sup>a</sup>	0.33 $\pm$ 0.17 <sup>b</sup>	0.39 $\pm$ 0.18 <sup>ab</sup>	0.37 $\pm$ 0.16 <sup>ab</sup>	0.41 $\pm$ 0.15 <sup>a</sup>
<b>Ileum</b>					
VH (mm)	0.68 $\pm$ 0.18 <sup>ab</sup>	0.67 $\pm$ 0.15 <sup>ab</sup>	0.71 $\pm$ 0.15 <sup>ab</sup>	0.64 $\pm$ 0.22 <sup>b</sup>	0.73 $\pm$ 0.12 <sup>a</sup>
VW (mm)	0.12 $\pm$ 0.04 <sup>b</sup>	0.12 $\pm$ 0.04 <sup>b</sup>	0.13 $\pm$ 0.04 <sup>ab</sup>	0.15 $\pm$ 0.07 <sup>a</sup>	0.13 $\pm$ 0.03 <sup>ab</sup>
CD (mm)	0.19 $\pm$ 0.06 <sup>b</sup>	0.21 $\pm$ 0.07 <sup>ab</sup>	0.23 $\pm$ 0.08 <sup>a</sup>	0.22 $\pm$ 0.06 <sup>ab</sup>	0.20 $\pm$ 0.06 <sup>b</sup>
VH:CD	3.89 $\pm$ 1.37 <sup>ab</sup>	3.46 $\pm$ 1.06 <sup>ab</sup>	3.47 $\pm$ 1.50 <sup>ab</sup>	3.21 $\pm$ 1.82 <sup>b</sup>	3.98 $\pm$ 1.33 <sup>a</sup>
VV (mm <sup>3</sup> )	0.0002 $\pm$ 0.000 <sup>b</sup>	0.0003 $\pm$ 0.0001 <sup>ab</sup>	0.0003 $\pm$ 0.0001 <sup>ab</sup>	0.0003 $\pm$ 0.0001 <sup>ab</sup>	0.0003 $\pm$ 0.00009 <sup>ab</sup>
VSA(mm <sup>2</sup> )	0.26 $\pm$ 0.12 <sup>b</sup>	0.28 $\pm$ 0.12 <sup>ab</sup>	0.30 $\pm$ 0.11 <sup>ab</sup>	0.32 $\pm$ 0.16 <sup>a</sup>	0.32 $\pm$ 0.10 <sup>a</sup>

Mean with different superscripts in a row are significantly different at P < 0.05. VH: Villus height; VW: Villus width; CD: crypt depth; VH: CD: Villus height to crypt depth ratio; VV: Villus volume; VSA: Villus surface area.

Intestinal mucosal architecture can be an indication of good gut health. A decrease in villus height implies an equivalent decrease in surface area for nutrient digestion whereas an increase in crypt depth implies a faster tissue turnover and a higher demand for new tissue. Both conditions can lead to increased gastrointestinal tract secretion, diarrhea, reduced diseases resistance, poor nutrient absorption, and lower overall performance <sup>(15)</sup>.

The results of this study show that plectasin supplementation did not affect the morphology of the small intestines as seen in the comparable villus height to crypt depth ratio, villus volume, and villus surface area values among the treatment groups. Similar to the results of this study, Jin *et al.*, <sup>(16)</sup> observed no difference in the villus height, villus width, crypt depth, and villus height to crypt depth



ratio of weanling pigs fed with an antimicrobial peptide derived from potato given at 0.25, 0.50, and 0.75 %. In contrast, plectasin supplementation in broilers at 100 mg/kg and 200 mg/kg led to increased villus height, reduced crypt depth in the jejunum, and increased villus height: crypt depth ratio compared to the negative control <sup>(17)</sup>. Similarly, Wan *et al.*, <sup>(18)</sup> obtained a uniform improvement in the villus height and villus height to crypt depth ratio in the duodenum, jejunum, and ileum of weaned pigs supplemented with recombinant plectasin (60 mg/kg) compared to the control. A study using another antimicrobial peptide cecropin at 2, 4, 6, and 8 ml/kg also showed improvement in villus height, crypt depth, and villus height to crypt depth ratio in broilers <sup>(19)</sup>.

The improvement in villus morphology due to AMPs may be related to their antibacterial function as pathogenic bacteria can produce a toxin in the gut that can cause inflammation of the mucosa, leading to morphologic changes like shortening of the villi and increase in crypt depth <sup>(20)</sup>. Antimicrobial peptides can effectively provide the necessary activity against pathogenic bacteria and the support for normal intestinal morphology and function. Additionally, AMP supplementation is believed to induce lower serum levels of D-lactate which increases intestinal permeability and boosts the efficiency of absorption and utilization of nutrients. The difference in the results observed in this study compared to similar investigations on AMPs may be attributed to the difference in the dose used and the kind of AMP used in the respective studies <sup>(21,22)</sup>.

For the evaluation of the selected blood values, supplementation with the different plectasin doses did not produce any difference in the serum levels of glucose, triglyceride, and cholesterol as compared to the control groups (Table 3).

**Table 3.** Mean (±SD) serum glucose, triglyceride, and cholesterol levels (mg/dl) of broiler chickens supplemented with plectasin.

Items	Treatment Groups				
	negative control	250 ppm enramycin (positive control)	150 ppm plectasin	300 ppm plectasin	450 ppm plectasin
Glucose	175.75 ± 24.29	173.83 ± 38.18	178.25 ± 22.72	171.17 ± 21.67	184 ± 28.02
Triglyceride	48.09 ± 11.65	55.91 ± 25.28	45.28 ± 8.33	41.55 ± 7.72	45.27 ± 14.45
Cholesterol	110 ± 10.69	111.5 ± 20.93	113.67 ± 15.11	104.08 ± 14.73	116.58 ± 17.15

No significant difference was observed among treatment groups.

Blood chemistry values can indicate infection or disease condition, or it may also be used as an index for nutrition and organ function <sup>(23)</sup>. However, not much research is available on the effects of AMPs on blood parameters. In the present study, selected blood serum parameters did not show any significant difference across treatment groups. A study by Dong *et al.*, <sup>(24)</sup> on the common carp (*Cyprinus carpio*) also showed no difference in glucose levels between the AMP treatment groups and the control. However, a significant decrease in cholesterol and triglyceride levels were seen in fish supplemented with higher doses of AMP. On the other hand, a similar study in piglets shows comparable levels of



glucose and triglyceride with the control whereas significantly lower cholesterol values were observed with AMP supplementation <sup>(25)</sup>. These two studies suggest that plectasin may be involved in the lipid metabolism of the body.

In the evaluation of the effect of plectasin on growth performance, all treatment groups were similar for the booster and starter phases. However, in the finisher phase, 150 ppm plectasin group had the highest ADG whereas the ADG in 450 ppm plectasin group was considerably lower than in the other groups. The ADFI was comparable in all treatments. A significant improvement in the FCR of birds in 150 ppm plectasin group was noted compared to the other groups (Table 4).

**Table 4.** Percent livability, mean ( $\pm$ SD) final weight, and ADG, ADFI, and FCR per feeding phase of broiler chickens supplemented with plectasin.

Items	Treatment Groups				
	negative control	250 ppm enramycin (positive control)	150 ppm plectasin	300 ppm plectasin	450 ppm plectasin
<b>Starter Phase</b>					
ADG (g)	20.24 $\pm$ 1.74	20.58 $\pm$ 1.36	20.70 $\pm$ 1.22	20.11 $\pm$ 1.10	20.52 $\pm$ 0.82
ADFI (g)	25.21 $\pm$ 1.12	24.72 $\pm$ 1.42	25.65 $\pm$ 0.34	24.59 $\pm$ 0.96	25.18 $\pm$ 0.59
FCR (g/g)	1.25 $\pm$ 0.08	1.20 $\pm$ 0.02	1.24 $\pm$ 0.07	1.22 $\pm$ 0.04	1.23 $\pm$ 0.06
<b>Grower Phase</b>					
ADG (g)	66.53 $\pm$ 2.89	67.22 $\pm$ 1.72	67.38 $\pm$ 1.56	68.54 $\pm$ 2.34	67.74 $\pm$ 3.11
ADFI (g)	88.51 $\pm$ 0.50	87.64 $\pm$ 2.34	89.72 $\pm$ 2.46	87.38 $\pm$ 2.49	88.43 $\pm$ 2.02
FCR (g/g)	1.33 $\pm$ 0.06	1.30 $\pm$ 0.02	1.33 $\pm$ 0.02	1.28 $\pm$ 0.02	1.31 $\pm$ 0.05
<b>Finisher Phase</b>					
ADG (g)	75.07 $\pm$ 4.61 <sup>ab</sup>	77.05 $\pm$ 6.26 <sup>ab</sup>	80.68 $\pm$ 5.40 <sup>a</sup>	73.43 $\pm$ 6.24 <sup>a</sup>	71.19 $\pm$ 3.20 <sup>b</sup>
ADFI (g)	149.02 $\pm$ 1.65	148.14 $\pm$ 3.92	151.91 $\pm$ 3.21	150.71 $\pm$ 7.93	149.69 $\pm$ 0.55
FCR (g/g)	1.99 $\pm$ 0.11 <sup>ab</sup>	1.93 $\pm$ 0.12 <sup>ab</sup>	1.89 $\pm$ 0.12 <sup>c</sup>	2.06 $\pm$ 0.15 <sup>ab</sup>	2.11 $\pm$ 0.10 <sup>a</sup>
Average Final Weight (kg)	1.94 $\pm$ 0.07	1.97 $\pm$ 0.07	2.02 $\pm$ 0.06	1.95 $\pm$ 0.09	1.92 $\pm$ 0.05
Livability (%)	95	98.33	98.33	90	98.33

Means with different superscripts in a row are significantly different ( $P < 0.05$ ).

The results of the finisher phase also showed a decreasing trend for ADG and a reducing trend for feed efficiency as the inclusion rate of plectasin increased. The average final weight of birds per treatment showed that 150 ppm plectasin group had the heaviest birds whereas 450 ppm plectasin group had the lightest birds. Additionally, percent livability for positif control group, 150 ppm plectasin group, and 450 ppm plectasin group was similar at 98.33 % whereas for negative control group and 300 ppm plectasin group it was lower at 95 % and 90 %, respectively (Table 4).

Various studies have reported an improvement in the growth performance of broiler chickens supplemented with AMPs <sup>(6)</sup>. This coincides with the result of the present study wherein higher ADG and better FCR were demonstrated in birds supplemented with 150-ppm plectasin. A recent study on recombinant plectasin by Ma *et al.*, <sup>(17)</sup> shows improved ADG and FCR in male broiler chickens. Similar results were obtained in broiler chickens supplemented with 20 mg/L, 30 mg/L, 150 mg/kg, and 200 mg/kg porcine antibacterial peptides in either drinking water or feeds <sup>(26)</sup>. Another study using recombinant plectasin in weaned piglets likewise resulted in improved ADG and FCR <sup>(18)</sup>.



The enhanced growth performance due to supplementation with AMPs has been attributed to improved nutrient digestibility and intestinal villus architecture<sup>(19,27)</sup>. The inhibition of the proliferation of pathogenic bacteria by AMPs (Guangdong Hinabiotech Co., Ltd., n.d.) could reduce the bacterial toxin-induced inflammation and adverse morphological changes in the gut such as shortening of the villi and increase in crypt depth thus preserving and improving villus architecture.

## 4. Conclusion

Plectasin supplementation at 150 ppm during the finisher phase improved the growth performance of broiler chickens by increasing the average daily gain and enhancing the feed conversion ratio. However, supplementation did not affect morphometric measurements of the small intestines and serum levels of glucose, triglycerides, and cholesterol. This study indicates that the antimicrobial peptide plectasin has beneficial effects on growth performance and improve nutrient utilization efficiency without disrupting normal physiological functions.

### Conflicts of interest statement

The authors declare no conflicts of interest.

### Data availability statement

The data will be provided upon request.

### Author contributions

Conceptualization: A.S.S. Favorito, M.G.V. Paraso, A.M.G.A Pajas, and J.F. dela Cruz, Data curation: A.S.S. Favorito. Formal analysis: J.F. dela Cruz, Funding acquisition: M.G.V. Paraso, Project management: M.G.V. Paraso. Methodology: A.S.S. Favorito, M.G.V. Paraso, A.M.G.A Pajas, and J.F. dela Cruz. Supervision: M.G.V. Paraso. Investigation: A.S.S. Favorito, M.G.V. Paraso, A.M.G.A Pajas, and J.F. dela Cruz. Visualization: A.S.S. Favorito, M.G.V. Paraso. Writing (original draft): A.S.S. Favorito, M.G.V. Paraso, A.M.G.A Pajas, and J.F. dela Cruz. Writing (proofreading and editing): A.S.S. Favorito, M.G.V. Paraso, A.M.G.A Pajas, and J.F. dela Cruz.

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