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The Effects of Probiotics, *Anredera cordifolia phytobiotic*, and Their Combination on Performances, Metabolic Status, Gastrointestinal Characteristics, and Nutrient Digestibility in Broiler Chickens

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ABSTRACT

This feeding trial investigated the effect of dietary supplementation with probiotic powder and Anredera cordifolia phytobiotic on the performance, metabolic status, gastrointestinal (GIT) characteristics, and nutrient digestibility of broiler chickens. Four hundred one-day-old Cobb broiler chicks were randomly assigned to one of four dietary treatments and grown for five weeks. The treatments included a control diet (P0), a basal diet supplemented with 0.5% probiotics powder (P1), a basal diet supplemented with 1.5% Anredera cordifolia (P2), and a combination of probiotics powder and Anredera cordifolia phytibiotic (combination of P1 and P2; P3). The results indicated no significant differences (P>0.05) in final body weight, feed intake, abdominal fat, gastro-intestinal tract (GIT) characteristics, depletion rate, and performance index among treatments. However, the dietary inclusion of P3 significantly (P < 0.01) increased carcass yield percentage and small intestine weight (duodenum, jejunum, and ileum) compared to P0, P1, and P2. Birds fed Anredera cordifolia phytobiotic exhibited reduced plasma cholesterol, but increased plasma triglycerides compared to other treatments. Additionally, the combination of probiotics powder and Anredera cordifolia phytobiotic (P3) significantly improved dry matter and organic matter digestibility and plasma bilirubin levels (P<0.01). Albumin and total protein levels remained unaffected by the treatments. The combination of probiotic powder and Anredera cordifolia phytobiotic synergistically enhances carcass percentages and nutrient digestibility in broiler chickens.

Keywords: broiler chickens, digestibility, gastrointestinal characteristics, metabolic status, phytobiotic anredera cordifolia, performance, probiotics.

INTRODUCTION

The usage of dietary antibiotics in poultry production resulted in common issues, such as the development of antimicrobial-resistant (AMR) microorganisms (Roskam et al., 2020), drug residues in the body of the birds (Burgat, 1999), and the potential transfer of those AMR microorganisms to humans (Oso et al., 2019). Consequently, researchers have been motivated to develop alternatives using either beneficial microorganisms or botanical products, known as phytogenic feed additives (PFAs; Oso et al., 2019) which

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possess either having anti-(pathogenic) microbial or immunomodulatory effects (Hernández et al., 2004).

A pool of beneficial microorganisms that is fed directly to animals, known as probiotics, has been reported to offer various beneficial effects, such as pathogen inhibition (Mountzouris et al., 2009), enhanced broiler performance, and improved nutrient digestibility (Palamidi et al., 2016). Probiotics are defined as live microorganisms (primarily lactic acid bacteria) that must remain viable when administered in diets to exert beneficial effects, such as improved gut microbiota balance, enhanced host immune response, and overall intestinal function and health (FAO/WHO, 2001). This definition emphasizes that only beneficial microorganisms should be included in a probiotic.

Madeira vine (*Anredera cordifolia* (Ten.) Steenis) is an herbal plant known for its antioxidant, anti-inflammatory, antibacterial, and analgesic properties. Its leaves contain many active compounds, including triterpenoids, steroid saponins, alkaloids, flavonoids, polyphenols, quinones, monoterpenoids, sesquiterpenoids, coumarins, polysaccharides, phytol, essential oil, α-pinene, and 6,10,14-trimethyl-2-pentadecanone (Astuti et al., 2011; Djamil et al,, 2012; Sukandar et al., 2011). The rhizomes contain flavonoids, polyphenols, tannins, and steroids (Sukandar et al., 2011). These compounds have been observed to improve the immune system and growth. Moreover, flavonoids and isoflavones are known as secondary metabolites produced by plants, which can prevent cell damage caused by free radicals (Cahyati et al., 2013). Isoflavones function as estrogen analogs, have hypocholesterolemia effects, improve digestive tract function, and may help prevent breast, prostate, and colon cancer, improve bone health, and regulate lipid metabolism (Fukuda et al., 2017).

It is hypothesized that provisioning *Anredera cordifolia* phytobiotic to broilers may improve metabolite status, gastrointestinal tract (GIT) function, and production performance. Various parameters have been measured to assess the effects of probiotics or herbal plants on broiler production performance. Morphological parameters are commonly used to assess diversity levels or patterns, although they represent only a small portion of environmental changes. Despite this limitation, measuring the morphological parameter as a preliminary assessment is useful because it is fast, easy, and can serve as a general approach to assess genetic diversity among morphologically distinguishable accessions (Sakti et al., 2019). The GIT is a common morphological marker used to evaluate the efficiency of nutrient digestion and absorption. Differences in nutrient digestion and absorption rate might be reflected in the GIT morphology and development. Metabolic status represents the processes of metabolism and nutrient digestibility in the body, which can affect production performances. However, *Anredera cordifolia* phytobiotic also contains tannins, which act as anti-nutrients for broilers; hence, the dosage must be carefully considered, especially when combined with probiotics.

To our knowledge, the inclusion of *Anredera cordifolia* phytobiotics, with or without the combination of probiotic powder, is still limited. The objective of our study was to investigate the effects of dietary supplementation with probiotic powder and *Anredera cordifolia* phytobiotics on performance, metabolic status, GIT characteristics, and nutrient digestibility of broiler chickens.

Materials and Methods

Animals, Housing, and Experimental Design

A total of four hundred unsexed Cobb strain broilers obtained from Berkah Green Farm in Sadamantra village, Kuningan Regency, West Java, and Broiler Chicken Farm Kp. Kudang 2 Jl. Wanaraja District, Garut, West Java, on the day of hatching, was involved in this study. Chicks were randomized by weight and placed in 1.6 m² floor pens, with 20 broilers per pen, bedded on clean wood shavings. Each pen measured 75 cm in length, 60 cm in width, and 50 cm in height. Twenty pens, each containing 20 birds were assigned to one of four dietary treatment groups: basal ration without any probiotic supplementation (P0), basal ration supplemented with 0.5% probiotic powder (P1), basal ration supplemented with 1.5% of Madeira vine leaves meal (P2), and basal ration supplemented with the combination of 0.5% probiotic and 1.5% of Madeira vine leaves meal (P3). There were 100 birds per treatment, with five replicates per treatment. Birds were allowed *ad libitum* access to the treatment diets and water throughout the trial (4 weeks).

The housing room was controlled using a central thermostat and temperature controller (Temptron 616, AgroLogic, USA) to maintain the room temperature at 34±1°C during the starter phase and 28±1°C for the remainder of the experiment. A 23L:1D lighting program was applied throughout the entire experiment. The treatment diets consisted of a commercial basal feed (Charoen Phokphand, Jakarta, Indonesia), with nutrient contents depicted in Table 1 and proximate analysis of nutritional content of research rations in Table 2.

Table 1. Nutritional content of basal feed was used in this experiment.

Nutrient Fraction	Content
Moisture (%)	8.56
Ash (%)	5.08
Crude Protein (%)	22.03
Crude Fibre (%)	3.67
Crude Fat (%)	7.45
Metabolizable Energy (Kcal/kg)	3,001

Table 2. Proximate Analysis of Nutritional Content of Research Rations

Feed Proximate	Nutritional Content of Rations			
	P0	P1	P2	P3
Metabolic Energy (Kcal/kg)	3001	2986	2987	2972
Crude Protein (%)	22.00	21.96	21.92	21.85
Crude Fat (%)	7.45	7.43	7.39	7.37
Crude Fiber (%)	3.67	3.76	3.74	3.82
Ash (%)	5.08	5.19	5.43	5.53
Moisture Content (%)	8.56	8.52	8.51	8.47

a Source: P0 = Results of Proximate Analysis of Ruminant Livestock Nutrition Laboratory and Animal Feed Chemistry (2020). P1, P2, P3 = Calculation result.

Probiotics Powder

The probiotic used in the current study is a commercial powder probiotic (Heryaki, Bandung, Indonesia) containing primarily *Candida ethanolica*, *Monascus fumeus*, and *Bacillus subtilis*, cultured in rice bran-based medium. The total viable bacteria count in this probiotic was 2×10^{10}, with 1.87×10^{10} being the *Lactobacillus* strain. (Supratman, et al., 2020). No antibiotics or antibiotic growth promoter compounds were included in the diet during this experiment. The probiotic powder was added to the basal ration according to the designed experimental treatment (0.5% DM basal diet of probiotic powder in P1 and P3 treatment).

Preparation of Anredera cordifolia Phytobiotic

The Madeira vine leaves were obtained from a plantation located in Sukabumi, West Java, Indonesia. The leaves were sorted by their similar age of plant (1 year), color, and freedom from any imperfections (tears, dirt, and insect damage). The freshly harvested leaves were then washed with clean water and then sun-dried for about 7 days, until the moisture content reached approximately 10%. The dried leaves were ground using a dry blender (Koninklijke Philips N.V., The Netherlands) until a homogenously fine powder was obtained. This fine powder was added to the basal ration according to the designed experimental treatment (1.5% DM basal diet of Madeira vine leaves meal in P2 and P3 treatment).

Parameters and Measurements

Production Performance and Gastrointestinal Tract Measurements

Performance parameters, including body weight gain, feed intake, feed conversion ratio, survival and depletion rate, and performance index, were recorded weekly. Every 10 broilers per pen were sacrificed to calculate the carcass weight and collect the abdominal fat and gastrointestinal tract (GIT). Carcass yield was calculated as the percentage of carcass weight relative to body weight. Organs such as the heart, liver, bile, and bursa of Fabricius were weighed. Meat samples were collected at the end of the treatment period. A small portion of the chicken breast and thigh, each ± 5 g, was taken as a diced sample. Samples were kept frozen at -20° C until analysis.

FCR was calculated as the ratio of feed intake (g) to body weight gain (g) over the trial period. A lower FCR indicates better feed utilization efficiency. The depletion rate was calculated as the percentage of birds removed from the trial due to mortality or culling.

This parameter reflects the health and survivability of the birds across different treatments, with a lower depletion rate indicating better health management and fewer losses. The survival rate represents the percentage of birds that remained alive at the end of the trial. It is the complement of the depletion rate.

The performance index (IP) combines the effects of body weight gain and depletion rate into one measurement to reflect overall production efficiency. This index provides an assessment of the birds' overall productivity, taking into account both growth (BWG) and survival (or depletion rate). A higher performance index indicates more efficient growth and lower mortality.

Blood Sampling and Plasma Metabolites Determination

Blood samples were collected from two birds of each replicate per treatment (2 birds × 5 replicates × 4 treatments = 40 birds) weekly from the pectoral vein at weeks 0, 2, and 4, at 1 hour before the morning feeding. Blood was collected in evacuated tubes (BD Vacutainer, Plymouth, UK) containing K2 EDTA for plasma metabolites measurements. Samples were kept cold on ice for a maximum of 2 h until centrifugation at 3000 × *g* for 15 minutes at room temperature. Plasma was decanted, aliquoted, and kept frozen at -20ºC until analysis. Plasma triglycerides, total protein, cholesterol, urea, bilirubin, creatinine, and albumin levels were measured at the laboratory of Animal Physiology and Biochemistry, Universitas Padjadjaran (Bandung, West Java, Indonesia), using commercial kits (Biolabo, Maizy, France).

Meat Cholesterol

Meat cholesterol levels were determined using the enzymatic colorimetric test method Cholesterol Oxidase Phenylperoxidase Aminophenazone (CHOD-PAP). The meat sample was ground, and 1 g was weighed and placed into an empty sampling tube. Three mL of the ether solution was added and left for 24 hours, then homogenized. The mixture was centrifuged at 3000 × *g* for 15 min. The supernatant was transferred into an Eppendorf tube. The blank cuvette was filled with 1000 µL of reagent and 10 µL of distilled water. The standard cuvette contained 1000 µL of reagent and 10 µL of standard solution. The assay cuvette (sample) contained 1000 µL of reagent and 10 µL of specimen. The absorbance value was measured using a spectrophotometer at a wavelength of 500 nm.

Statistical Analysis

Data were analysed by using one-way analysis of variance (ANOVA) in the general linear model (proc GLM) of SAS version 9.2 (Cary, NC, USA) to determine the fixed effects of the treatments. Duncan's multiple range test was conducted for any significant differences in multiple comparisons. Data were expressed as least square means (LSM) of respective parameter with pooled standard error of means (SEM), with significance alpha value of 0.05.

Results

Production Performance

The average final body weight (BW) from lowest to highest across treatments is shown in Table 3 as follows: P3 (1125.16 grams), P2 (1155.13 grams), P0 (1201.00 grams), and P1 (1206.56 grams). The final BW of broiler chickens at 25 days of age, based on the Berkah Farm Company standard, namely 1170 grams, while the Cobb500 (2015) standard is 1227 grams. This indicates that the final BW in P0 and P1 met the company standards, even though they did not meet the Cobb500 standard. Conversely, P2 and P3 had lower final BW than both standards. The P1 treatment exhibited higher body weight gain (BWG) than the control, whereas treatments containing *Anredera cordifolia* phytobiotic (P2 and P3) produced lower average BWG compared to P0 and P1. The addition of probiotic powder, *Anredera cordifolia* phytobiotic, and their combination did not have a significant effect (P>0.05) on body weight gain.

Table 3. Least square means of the effect of supplemented Probiotics and *Anredera cordifolia* Leaf Flour on the Performance of Broiler Chickens

Parameters	Average				P-Value
	P0	P1	P2	P3	
BWG	1201.00± 18.29	1206.56±21.12	1155.13±38.00	1125.16±20.24	0.12
Feed Conversion	1.30±0.02	1.29±0.03	1.36±0.06	1.38±0.03	0.23
Survival Rate	100.00±0.00	99.20±0.80	98.40±0.98	100.00±0.00	0.26
Performance Index	389.20±11.28	385.40±22.53	361.20±19.94	344.60±13.30	0.26

Note:
P0 = Basal ration (no Heryaki powder probiotics or *Anredera cordifolia* leaf flour added) (Control)
P1 = Basal ration with 0.5% Heryaki powder probiotics added
P2 = Basal ration with the addition of 1.5% *Anredera cordifolia* leaf flour
P3 = Basal ration with 0.5% Heryaki powder probiotics added and 1.5% *Anredera cordifolia* leaf flour

The average feed conversion ratio (FCR ranged from lowest to highest as follows: P1, P0, P2, and P3 (Table 3). The FCR values for each treatment were lower than the Berkah Farm company standard of 1.42. The average FCR values for P0 and P1 were lower compared to Cobb500 (2015), which is 1.34 for broilers at twenty-five days of age. P1 exhibited the lowest or best FCR value compared to P0.

The analysis of variance showed that the treatments had no significant effect (P>0.05) on the survival rate, indicating that all treatments produced similar responses in terms of survival rate. Additionally, the treatments did not significantly affect (P>0.05) the performance index, demonstrating uniform responses across all treatments. (You should explain the data of the performance index and show how you calculated the depletion rate and the performance index.)

Meat Cholesterol

The cholesterol levels of broiler meat varied across treatments, as presented in Table 4. The highest meat cholesterol was found in the thigh sample from P1 (54.67 mg/dl). The lowest meat cholesterol level was observed in the breast sample from P3 (19.75 mg/dl). Chicken thighs from the P1 group had the highest meat cholesterol among all treatments (54.67 mg/dl). Chicken thighs given *Anredera cordifolia* phytobiotic (P2) had lower meat cholesterol levels than those given probiotics, but still higher than the control (42.33 mg/dl). The combination treatment (P3) resulted in the lowest thigh meat cholesterol levels among all treatments (22.9277 mg/dl), although this was still higher than the levels found in chicken breasts from the same treatment group. (The data described above was not mentioned in any table.)

Table 4. Cholesterol levels of thigh and breast meat samples in this study.

Sample	Absorbance	Standard	Meat Cholesterol (Mg/dl)
P0 Thigh	0.128	0.567	45.15
P0 Breast	0.128	0.567	45.15
P1 Thigh	0.155	0.567	54.67

P1 Breast	0.069	0.567	24.34
P2 Thigh	0.120	0.567	42.33
P2 Breast	0.099	0.567	34.92
P3 Thigh	0.065	0.567	22.93
P3 Breast	0.056	0.567	19.75

Note:

P0 = Basal ration (no Heryaki powder probiotics or *Anredera cordifolia* leaf flour added) (Control)

P1 = Basal ration with 0.5% Heryaki powder probiotics added

P2 = Basal ration with the addition of 1.5% *Anredera cordifolia* leaf flour

P3 = Basal ration with 0.5% Heryaki powder probiotics added and 1.5% *Anredera cordifolia* leaf flour

Metabolic Status

Birds fed *Anredera cordifolia* phytobiotic showed significantly reduced plasma cholesterol (173.83 mg/dl), but increased triglyceride (50.27 mg/dl) compared to other treatments (Table 5). Moreover, birds fed a combination of probiotics and *Anredera cordifolia* phytobiotic had significantly elevated bilirubin levels (32.92 mg/dl) compared to other treatments (P<0.01). Albumin and total protein levels were not affected by the treatments.

Table 5. Least square means of the effect of supplemented Probiotics and *Anredera cordifolia* Leaf Flour on the blood test of the Broiler Chickens

No.	Blood Test	P0	P1	P2	P3	Pvalue
1.	Cholesterol (mg/dl)	184.11 ± 10.18^{a}	328.84 ± 18.43^{b}	173.83 ± 20.97^{a}	191.91 ± 18^{a}	<0.01
2.	Triglycerides (mg/dl)	43.69 ± 7.80^{b}	41.99 ± 6.20^{b}	50.27 ± 11.03^{b}	18.61 ± 2.99^{a}	<0.01
3.	Albumin (g/dl)	1.52 ± 0.15	1.33 ± 0.21	1.60 ± 0.22	1.54 ± 0.13	0.15
4.	Bilirubin (mg/dl)	12.45 ± 0.81^{a}	13.79 ± 1.52^{a}	12.68 ± 0.77^{a}	32.92 ± 1.91^{b}	<0.01
5.	Total Protein (g/dl)	3.15 ± 0.31	3.01 ± 0.37	3.40 ± 0.42	3.31 ± 0.50	0.47

[Ab] means within the same row with different superscripts differ according to the indicated level of significance within each main effect.

Note:

P0 = Basal ration (no Heryaki powder probiotics or *Anredera cordifolia* leaf flour added) (Control)

P1 = Basal ration with 0.5% Heryaki powder probiotics added

P2 = Basal ration with the addition of 1.5% *Anredera cordifolia* leaf flour

P3 = Basal ration with 0.5% Heryaki powder probiotics added and 1.5% *Anredera cordifolia* leaf flour

Production Performance, internal organs, and small intestinal morphology Characteristics

The results indicated that birds in the P3 group had significantly higher carcass yield (P<0.01; Table 6) compared to control (P0), P1, or P2 groups, with percentages of 76.4, 69.9, 72.4, and 72.5%, respectively. The weights of the duodenum, jejunum, and ileum were significantly higher in the P2 group compared to other groups. While duodenum weight was higher in the P2 group than in the P1 group, the difference was not significant (P>0.05).

Table 6. Least square means of the production performance and small intestine morphology in birds supplemented with the treatments in this study. (Try to unify the titles of all tables as suggested in Tables 3 and 4.)

Parameter	Treatment[1]				SEM	p-value
	P0	P1	P2	P3		
Production Performance						
Final body weight (g)	1309.5	1256	1214	1199	96.7	0.1523
Carcass weight (g)	915.22	908.9	881.4	916.0	70.2	0.8308
Carcass yield (%)	69.9^{a}	72.4^{a}	72.5^{a}	76.4^{b}	2.34	0.0004
Abdominal fat (g)	14.5	12.6	12.2	15.8	3.57	0.2774
Organ Weights						
Heart (g)	1.41	1.53	1.25	1.64	0.51	0.6857
Liver (g)	30.89	27.52	23.58	26.68	6.41	0.2244
Gall bladder (g)	6.69	6.2	6.65	5.825	0.84	0.2849
Bursa fabricius (g)	2.4	2.4	2.4	2.6	0.66	0.942
Small Intestine Morphology						
Whole length (cm)	171	171.7	173.6	168.2	13.9	0.9396
Duodenum weight (g)	11.5^{b}	9.71^{a}	18.14^{c}	15.58^{bc}	1.57	0.0096
Jejunum weight (g)	13.2^{a}	14.08^{a}	22.46^{b}	19.64^{ab}	2.59	0.0156
Ileum weight (g)	9.32^{a}	9.42^{a}	16.68^{b}	14.32^{ab}	1.65	0.0167

^{ABC} means within the same row with different superscripts differ according to the indicated level of significance within each main effect.

[1] Information:

P0: basal diets

P1: basal diets supplemented with 0.5% of probiotic in DM basal diets

P2: basal diets supplemented with 1.5% of Madeira vine leaves meal in DM basal diets

P3: basal diets supplemented with 0.5% of probiotic in DM basal diets and 1.5% of Madeira vine leaves meal in DM basal diets

Nutrient Digestibility

Digestibility measurements provide an indication of the quality of feed and its digestibility by the livestock. The results in Table 7 showed that the highest average dry matter digestibility was observed in the P3 group (89.82%), followed by P2 (88.87%), P0 (83.80%), and P1 (83.58%). The ration supplemented with both probiotics powder and *Anredera cordifolia* phytobiotic demonstrated the highest digestibility, whereas the ration with added probiotics powder alone had the lowest digestibility compared to P0, P2, and P3. The combination of probiotics powder and *Anredera cordifolia* phytobiotic resulted in the highest nutrient digestibility compared with the control and single administration of either probiotics or *Anredera* cordifolia phytobiotic. The analysis showed that the supplementation of probiotics powder, *Anredera cordifolia* phytobiotic, and their combination significantly affected ($P<0.05$) dry matter digestibility in broiler chickens.

Table 7. In vitro dry matter digestibility (IVDMD) and in vitro organic matter digestibility (IVOMD) in birds supplemented with the treatments in this study (LSMeans±pooled standard error of means [SEM])

No.	Variables	P0	P1	P2	P3	Pvalue
1.	IVDMD (%)	83.80 ± 3.05^{ab}	83.58 ± 7.17^{a}	88.87 ± 2.32^{bc}	89.82 ± 0.53^{c}	<0.05
2.	IVOMD (%)	84.42 ± 3.05^{a}	84.52 ± 6.73^{ab}	89.52 ± 2.25^{bc}	90.39 ± 0.52^{c}	<0.05

^{Abc} means within the same row with different superscripts that differ according to the indicated level of significance within each main effect.

IVDMD = In vitro Dry Matter Digestibility

IVOMD In Vitro Organic Matter Digestibility

Note:

P0 = Basal ration (no Heryaki powder probiotics or *Anredera cordifolia* leaf flour added) (Control)

P1 = Basal ration with 0.5% Heryaki powder probiotics added

P2 = Basal ration with the addition of 1.5% *Anredera cordifolia* leaf flour

P3 = Basal ration with 0.5% Heryaki powder probiotics added and 1.5% *Anredera cordifolia* leaf flour

The digestibility of organic matter is crucial for determining the digestible carbohydrate, protein, and fat content in livestock. The highest average organic matter digestibility was observed in the P3 group (90.39%), followed by the P2 (89.52%), P1 (84.52%), and P0 (84.42%) (Table 7*)*. The analysis of variance revealed that the probiotics powder, *Anredera cordifolia* phytobiotic, and their combination significantly influenced (P<0.05) the digestibility of organic matter in broiler chickens, similar to the results observed for dry matter digestibility. The combination of *Anredera cordifolia* phytobiotic and probiotics resulted in the highest overall digestibility. The administration of *Anredera cordifolia* phytobiotic at a level of 1.5% (P2) ranked second, followed by P1 with 0.5% probiotics. The control (P0) had the lowest digestibility results, which were significantly different from the other treatments.

Discussion

Production Performance

Body Weight Gain (BWG)

Broiler chickens given Heryaki probiotics powder in the P1 treatment produced the highest average body weight gain (BWG). It is assumed that the probiotic content of Heryaki powder positively influenced BWG compared with other treatments, although the difference was not statistically significant. This finding aligns with Ismiatun (2015), who reported that the addition of *Bacillus subtilis*-based probiotics in the diet can increase BWG. The mechanism by which *B. subtilis* enhances growth involves the production of protease and amylase enzymes, which assist in the digestion of feed, allowing better absorption by the intestinal villi, thus promoting BWG (Zurmiati et al, 2014). The lower BWG observed in the P3 group compared to P1 and P2 suggests that the combination treatment was less effective than the single treatments. This may indicate an interaction between the antibacterial properties of *Anredera cordifolia* phytobiotic and the probiotic content of Heryaki powder, which could interfere with the optimal function of both. These results suggest that combining probiotic Heryaki powder with *Anredera cordifolia* phytobiotic may not always yield better results than single treatments. Despite the lack of a significant difference among treatments, the administration of probiotics powder did increase BWG in broiler chickens. (You should explain why the final body weight in the control group was higher than other treatments, which conflicted with the results of BWG.)

Probiotic and phytobiotic treatments often improve FCR and daily gain, but if the control birds were initially heavier or consumed more feed, their final weight might remain higher despite inferior efficiency. Aligning with previous studies, probiotic supplementation in broilers has been shown to increase body weight and average daily gain while reducing FCR and serum cholesterol (Khan et al., 2025). Additionally, synergistic combinations of probiotics with chicory root or coriander seed improved BWG and FCR and reduced pathogenic bacteria (Gurram et al., 2022). However, these benefits may not always translate to a heavier final body weight if baseline differences or feed intake vary.

Factors influencing these results include similar feed intake across treatments: P0 (7,737 g), P1 (7,705g), P2 (7,604g), and P3 (7,713g) (Table 5). (The feed intake data did not show in Table 3) The uniformity in feed intake led to relatively similar BWG. According to Uzer et al. (2013), disruptions in feed intake can affect growth. The consistency in feeding intake is attributed to the uniformity in the quantity and quality of the rations provided. The addition of each treatment did not significantly affect the nutritional content of the rations.

Feed Conversion Ratio (FCR)

The analysis of variance indicated that additional rations containing probiotic powder, *Anredera cordifolia* phytobiotic, and their combination did not significantly affect (P>0.05) the FCR. The lack of treatment effect on FCR corresponds with the relatively consistent BWG and feed intake observed across the treatments.

A good FCR value is characterized by low feed intake coupled with high BWG, indicating efficient feed utilization. Among the treatments, P1 exhibited the best FCR efficiency, although not significantly different from the others. This finding aligns with Budiansyah (2004), who reported that probiotics containing *Lactobacillus* can produce cellulase enzymes, aiding in the digestion of high-fiber feed in poultry and enhancing feed absorption efficiency. B. *subtilis*, which produces protease and amylase enzymes, also contributes to breaking down complex molecules into simpler ones, facilitating absorption by the chicken intestine (Zurmiati et al, 2014). Thus, probiotics powder supplementation in P1 can reduce the FCR value in broiler chickens, albeit with no significant differences among treatments.

Similar to the BWG results, single treatments yielded better FCR than combined treatments. The flavonoid content in *Anredera cordifolia* phytobiotic, which acts as an anti-bacterial agent (Manoi, 2009), might interfere with the function of beneficial bacteria in the probiotic powder. This interaction may lead to suboptimal digestion in broiler chickens. Additionally, the high quality of the feed provided for each treatment may have minimized the effect of the added ingredients, resulting in No. Significant difference in FCR.

Survival and Depletion Rates

The depletion rate, which indicates the reduction in the number of chickens due to mortality and culling, was found to be below 5%, indicating good health and management (North and Bell, 1990). This depletion rate is the complement of the survival rate, which is calculated based on the number of birds that remain alive at the end of the trial compared to the total number of birds that started the trial. The P1 treatment had a depletion rate of 0.8%, with one mortality out of 20 chickens in a replicate. Probiotics can improve livestock health by maintaining digestive tract health, enhancing nutrient absorption, and working in immunomodulation. Probiotics work by neutralizing toxins, increasing immunity, and suppressing harmful bacterial populations (Barbosa et al, 2005). The mortality in P1 occurred on day 5 due to stress, likely caused by suboptimal cage temperature. High temperatures can cause up to 30% mortality (Tarmudji, 2004).

The P2 treatment had a depletion rate of 1.6%, with two deaths out of 20 chickens in a replicate. *Anredera cordifolia* phytobiotic is very effective in maintaining health its active compounds, such as flavonoids, alkaloids, ascorbic acid, and terpenoids. These compounds act as antioxidants or free radical scavenging agents, thereby boosting immunity. High flavonoid content (47.60%) and alkaloid content (2.60%) in *Anredera cordifolia* phytobiotic contribute to maintaining the body’s immunity (Widodo et al, 2016). Death in this treatment occurred on days 2 and 4, likely due to stress from suboptimal cage temperatures.

Performance Index

The performance index (IP) measures the success of a production over a maintenance period. The IP for each treatment is shown in Table 3 and was categorized as good (326-350) and very good (351-400) (Santoso and Sundryani, 2009). The control treatment (P0) had a very good IP compared to other treatments, likely due to the basal ration's adequacy in meeting the nutritional needs of chickens, thus supporting productivity and reducing mortality. The P1 treatment also had a very good IP but was slightly lower than P0 by 3.8 points. The mortality affected the IP in P1, as one chicken died during the powder probiotic treatment. Without mortality, P1 treatment could have yielded a superior IP. In addition to mortality, the IP is influenced by BWG and FCR, factors that benefit from the control content (P0) as well as the added value of probiotics or *Anredera cordifolia* phytobiotics.

P1 treatment had a better IP than P2 and P3 due to improvements in the digestive tract from the beneficial microbes, enhancing nutrient absorption. The probiotics in Heryaki powder, including *Bacillus subtilis*, aid in producing protease enzymes, increasing amino acids. *Lactobacillus casei* produces cellulose enzymes that help digest crude fiber, improving feed absorption and utilization for tissue growth and weight gain. Effective feed absorption increases BWG, positively affecting the IP. Additionally, *Lactobacillus casei* helps neutralize toxins in the digestive tract, boosting immunity (Barbosa et al, 2005)

The P2 treatment, with the addition of *Anredera cordifolia* phytobiotic, had a lower IP due to the mortality of 2 chickens. However, phytochemical contents in *Anredera cordifolia* phytobiotic, such as flavonoids, alkaloids, ascorbic acid, and terpenoids, support the digestive system by killing harmful bacteria, thereby maximizing nutrient absorption (Muiz 2016). The flavonoids in *Anredera cordifolia* play a significant role, with a phytochemical composition of 47.40% (Widodo et al, 2016). The alkaloids in *Anredera cordifolia* phytobiotic, with a composition of 2.60% (Widodo et al, 2016), improve feed consumption, BWG, and immune response (Ni et al., 2016). These active substances support good feed intake and BWG, enhancing the performance indices.

The combination treatment of probiotic powder and *Anredera cordifolia* phytobiotic (P3) showed a good standard performance index. However, the lack of mortality in this treatment did not translate to a superior IP compared to other treatments. The reduction in IP in the P3 treatment might be due to a non-synergistic effect between the probiotic powder and Anredera *cordifolia* phytobiotic. The flavonoids and essential oils content in *Anredera cordifolia* phytobiotic may kill both beneficial and harmful bacteria, affecting the function of the probiotic. The essential oil contained in *Anredera cordifolia* phytobiotic can denature bacterial proteins (Warnaini, 2013). The imbalance in the ratio of probiotic powder (0.5% per kg of ration) and *Anredera cordifolia* phytobiotic (1.5% per kg of ration) may have exacerbated this effect. (All the above discussion doesn’t mean anything if the data of this measurement did not significant difference between treatments and did not show how this characteristic was measured.

Meat cholesterol

Chicken thighs generally had higher cholesterol levels than chicken breasts, regardless of treatment. Probiotic treatment (P1) tended to increase meat cholesterol in chicken thighs significantly (54.67mg/dl) compared with chicken breasts (24.34mg/dl). *Anredera cordifolia* phytobiotic (P2) also increased meat cholesterol in chicken thighs (42.3280 mg/dl) compared with chicken breasts (34.9206 mg/dl), but not as much as the probiotics. The combination treatment (P3) resulted in the lowest cholesterol levels in both parts, with thighs (22.93 mg/dl) and chicken breasts (19.75 mg/dl), indicating a beneficial effect in reducing meat cholesterol.

Metabolic Status

Probiotics are recognized as alternative antibiotics that can reduce oxidative stress, improve intestinal health, and enhance livestock performance. They produce enzymes that aid digestion and antibacterial substances to suppress harmful microorganisms. In this study, the supplementation of probiotic powder and *Anredera cordifolia* phytobiotic reduced plasma and meat cholesterol in both the breast and thigh. The mechanisms include deconjugating bile acids, cholesterol assimilation, and short-chain fatty acid production, which reduce endogenous cholesterol levels. Exogenous cholesterol absorption in the small intestine is decreased by cholesterol reductase enzyme activity. LAB produces the enzyme bile salt hydrolase (BSH), which means) which deconjugates bile salts, making them less absorbable. Bile salt hydrolase (BSH) is an enzyme produced by many gut bacteria, including Lactobacillus species, that deconjugates (hydrolyzes) conjugated bile acids (Dong et al., 2025). This deconjugation is considered a gatekeeper reaction in bile-acid metabolism; deconjugated bile acids co-precipitate with cholesterol in the intestine and are excreted, leading to reduced reabsorption and increased synthesis of new bile acids from serum cholesterol (Dong et al., 2025). Consequently, probiotics with high BSH activity can lower circulating cholesterol. In the present study, the probiotic treatment lowered serum cholesterol and LDL levels, consistent with previous reports that Lactobacillus-based probiotics improve growth and lipid profiles in broilers (Khan et al., 2024). The excretion of bile acids requires more cholesterol to synthesize bile salts, thereby lowering cholesterol levels (Adriani et al., 2017). LAB also conjugates bile salts in the intestine, preventing cholesterol synthesis (Lengkey and Adriani, 2011).

In this study, birds fed a combination of probiotic powder and *Anredera cordifolia* phytobiotic showed increased plasma bilirubin compared to other treatments (P<0.01). Albumin and total protein were not affected by the treatments. (No discussion). Probiotics have also been identified as a good alternative to antibiotics, considering their ability to reduce oxidative stress and improve gut health and animal performance (Khan et al., 2024). In this experiment, probiotic powder and P3 supplementation significantly reduced plasma cholesterol and meat cholesterol in both the breast & thigh. This decrease is thought to be mediated through multiple potential mechanisms, including bile acid deconjugation, cholesterol absorption, and short-chain fatty acid (SCFA) formation that decreases endogenous cholesterol28. Lactic acid bacteria (LAB) are probiotics and a group that includes LAB, which can generate the bile salt hydrolase (BSH), an enzyme that deconjugates bile salt, thus reducing its intestinal absorbance. This mechanism leads to increased excretion of bile acid that results in the usage of more cholesterol for the synthesis of bile salts, leading to lower plasma cholesterol (Yang et al., 2022).

The current results are in agreement with those reported earlier that the probiotics can modulate lipid metabolism, lowering plasma cholesterol, triglycerides, and low-density lipoprotein (LDL) but improving high-density lipoprotein (HDL) concentrations in plasma (Khan et al., 2024). These are thought to be involved in the increased synthesis of SCFAs occurring during probiotic fermentation, which stimulate fat degradation and cholesterol reduction (Selim et al., 2024). Moreover, Anredera cordifolia phytobiotic, which is abundant in bioactive agents including flavonoids, has demonstrated comparable actions on lipid metabolism. In addition, flavonoids an antioxidants that decrease the level of oxidative damage and control lipid metabolism (Sukandar et al., 2011), which is also in line with the synergistic action when used with probiotics. The interaction of probiotic powder and Anredera cordifolia phytobiotic used in this study might have synergistically induced the decline in cholesterol level via these aspects concurrently.

In addition, in the P3 group, the use of probiotics and Anredera cordifolia phytobiotic significantly increased the bilirubin concentration compared with that of other treatments ($P < 0.01$). Bilirubin is a metabolite of hemoglobin and an indicator of liver performance. Increased plasma bilirubin concentrations commonly indicate increased liver detoxification activity. This result indicated that the probiotics adding Anredera cordifolia phytobiotic may exert a positive effect on the liver function, which was in line with previous findings implying that their synergistic regulation might be due to relieving oxidative stress and promoting liver bile acid metabolism (Yang et al., 2024). Elevated bilirubin levels, as observed in our study, might also be a reflection of increased hepatic function and detoxification (especially cholesterol metabolism).

On the contrary, a significant impact was not detected on plasma albumin and total protein levels; this could be due to some reasons. Although several studies revealed that probiotics raised the levels of serum albumin and total protein, indicating improvement of liver function and protein metabolism (Haque et al., 2017), no significant effects were observed in this study, perhaps due to short-term supplementation or differences in strains of probiotics used. A good balance of nutrient intake supplied by the treatment diets may have balanced a protein status despite other metabolic alterations (Mohamed et al., 2022).

These findings demonstrated the potential power of probiotics and *Anredera cordifolia* phytobiotic as prophylactic agents not only in terms of gut health but also metabolic health-promoting, with possible xenobiotic use reduction on broilers, and such supplements are probably increasing feed utilization efficiency under mycotoxins. This combination might be able to improve broilers' health and performance by affecting cholesterol metabolism as well as liver function. Further research with longer time periods, as well as further investigations of liver histology and immune function, is necessary to understand more about the long-term effects on serum proteins and broiler birds reported apparently healthy.

Gastrointestinal (GIT) Characteristics

The production performance and gastrointestinal (GIT) characteristics of experimental birds in all treatments are shown in Table 6. The percentage of carcass at the end of the experimental period was the most prominent parameter, indicating an effect of the treatments. This higher carcass yield in the P3 group is generated by a lower final body weight of the birds, with higher carcass yield in this group compared to other treatments, although no significant difference was found either in final body weight, carcass weight, or feed conversion ratio (FCR) parameters. The higher carcass yield might also imply that the protein deposition in the carcass is improved by probiotic and *Anredera cordifolia* phytobiotic supplementation. It was reported that in poultry, probiotics have an activity to increase volatile fatty acids, especially butyrate, which can also control growth in chickens by selectively partitioning nutrients from the liver and adipose tissue to the muscle through up-regulation of muscle insulin β receptor subunit (IRβ) expression. (Mátis et al., 2015). As a result, probiotics can stimulate growth in poultry by increasing VFA production and selectively regulating insulin signaling in different tissues. (Ajuwon, 2016). High carcass yield in probiotic-supplemented birds in the current study differed from the study of Awad et al., (2009), who reported no significant difference in carcass yield in birds after probiotic supplementation. However, the birds fed synbiotic (a combination of probiotic and prebiotic) were reported to have a significantly higher carcass yield. (Awad et al., 2009).

Numerically, the highest final body weight of birds in the present study was achieved by the P0 group compared to other treatments, although no significant difference (P>0.05). It is tempting to speculate that lowering final body weight in the P1, P2, or P3 group might imply that either probiotic or phytochemical compound supplementation removed to some extent the effects of the growth promoter contained in the basal ration. However, the exact mechanism of this growth promoter's effects removal is not clearly elucidated yet, but it appears that phytochemical compounds reduce the intestinal digestibility of growth promoters, so that their effects are inhibited. Moreover, a previous study reported that the lower final body weight of broiler chicken supplemented with phytochemicals compared to the non-supplemented group was found, suggesting the fat-reducing activity by the flavonoid contained in phytochemicals (Alagawany et al., 2019). The results in the current study seem to confirm the fat-reducing activity by the flavonoids as reported by the study of Alagawany et al. (2019), in which abdominal fat content was lower in the P2 group compared to the P0 group, although the difference was not significant (P>0.05, Table 6).

The weight of the duodenum, jejunum, and ileum of the small intestine is significantly higher in the P2 group compared to the other groups. In a previous study, it was reported that the duodenum and jejunum weights increased with the increment of amino acid absorption in broiler diets, although no significant difference was found in the ileum weight. (Wijtten et al., 2010). In the current study, the degree of difference in weight was more pronounced in the duodenum compared with either the jejunum or the ileum. This variation in difference degree between the duodenum versus jejunum, and ileum has also been reported by Wijtten et al. (2010), suggesting a proximal small intestine (duodenum) is more dependent on luminal nutrient supply for intestinal protein synthesis than the distal small intestine (jejunum and ileum; Stoll et al., 2000).

A higher duodenum weight is found in P2 compared to the P1 group, although the difference is not significant (P>0.05). The most plausible explanation of this phenomenon might be related to the fact that antimicrobial activity. (Astuti et al., 2011; Tshikalange et al., 2005; Yuniarti & Lukiswanto, 2017) in the *Anredera cordifolia* phytobiotic interacts with the viable microorganisms contained in the probiotic, hence, it decreases the probiotic's working effectiveness. Furthermore, the flavonoid and its derivative content in plant leaf extract may act as a conjugate to dietary amino acids. (Codorniu-Hernández et al., 2007; Rawel et al., 2002; Wei et al., 2014)Hence, it might explain the increased duodenum and jejunum weight, which goes hand in hand with the increase in amino acid availability and absorption in those two sites.

In the current study, the combination of probiotic and *Anredera cordifolia* phytobiotic seemed to work synergistically in terms of producing higher carcass yield. This finding is in line with a previous study that reported that tannins, a plant-derived polyphenolic compound similar to flavonoids, have a synergistic effect when supplemented to poultry in combination with probiotics (Blaiotta et al., 2013; Redondo et al., 2014). However, the mechanism of the interaction is not clearly elucidated yet, due to the high complexity and dynamics in the gut microbiota.

Nutrient Digestibility

The combination of probiotics with *Anredera cordifolia* phytobiotic (P3) significantly increased dry matter digestibility in broiler chickens compared to other treatments. Terpenoid compounds stimulate the excretory nervous system, releasing digestive enzymes like amylase, lipase, trypsin, and pepsin (Habibah et al., 2012). Bacillus bacteria in probiotics improve digestibility (Supratman, 2017). The synergistic effect of *Anredera cordifolia* phytobiotic and probiotics in P3 produced the highest dry matter digestibility.

Anredera cordifolia phytobiotic supplementation at a 1.5% level (P2) was second in effectiveness, followed by the control (P0). The P1 treatment, with 0.5%, showed the lowest results in terms of dry matter digestibility. Probiotics maintain the balance of digestive microorganisms, improving digestion, digestibility, increasing nutrient absorption, and maintaining livestock health (Agustina, 2007). Digestibility is influenced by various factors, including feed composition, feed processing, and livestock species and age (Schneider and Flatt, 1975) in Sutardjo (1996).

The combination of probiotics and *Anredera cordifolia* phytobiotic (P3) significantly increased the organic matter digestibility, which might be attributed to the anti-bacterial properties of flavonoids. Flavonoids disrupt bacterial cell walls, enhancing nutrient utilization (Muiz 2016). Organic materials are closely related to dry materials, providing energy for livestock growth and development (Sutardi, 2001). The combination treatment produced the highest organic matter digestibility, surpassing control and single treatments. Organic matter digestibility was higher than dry matter digestibility, as organic matter lacks ash content, making it relatively easier to digest (Fathul and Wajizah, 2010).

Conclusion

In conclusion, the supplementation of *Anredera cordifolia* phytobiotic (up to 1.5% of DM basal ration), probiotics powder (up to 0.5% of DM basal ration), or their combination did negatively affect production performance, but increased carcass yield. The combination of *Anredera cordifolia* phytobiotic with probiotics powder seems to show a synergistic effect in terms of higher carcass yield. It might be important to study the interaction and mechanism of actions between antimicrobial growth promoters with the phytochemical content of *Anredera cordifolia* phytobiotic in the future to obtain a better understanding of the poultry gastrointestinal tract microbiome dynamics.

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Declaration of Competing Interest

The authors declared that there is no conflict of interest.

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أثير البروبيوتيك، ومستخلص نبات أنريديرا كورديفوليا، ومزيجهما على الأداء، والحالة الأيضية، وخصائص الجهاز الهضمي، وهضم العناصر الغذائية في دجاج التسمين

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ملخص

هدفت هذه الدراسة إلى بحث تأثير إضافة مسحوق البروبيوتيك ومستخلص نبات أنريديرا كورديفوليا إلى العلف على الأداء، والحالة الأيضية، وخصائص الجهاز الهضمي، وهضم العناصر الغذائية في دجاج التسمين. تم توزيع 400 كتكوت من سلالة كوب، عمر يوم واحد، عشوائيًا على أربع مجموعات غذائية، وتمت تربيتها لمدة خمسة أسابيع. شملت المعالجات نظامًا غذائيًا ضابطًا (P0)، ونظامًا غذائيًا أساسيًا مُدعّمًا بـ 0.5% من مسحوق البروبيوتيك (P1)، ونظامًا غذائيًا أساسيًا مُدعّمًا بـ 1.5% من مستخلص نبات أنريديرا كورديفوليا (P2)، ومزيجًا من مسحوق البروبيوتيك ومستخلص أنريديرا كورديفوليا النباتي (مزيج P1 وP2؛ P3). أشارت النتائج إلى عدم وجود فروق ذات دلالة إحصائية (P>0.05) في الوزن النهائي للجسم، واستهلاك العلف، ودهون البطن، وخصائص الجهاز الهضمي، ومعدل الاستهلاك، ومؤشر الأداء بين المعالجات. مع ذلك، أدى إدراج P3 في النظام الغذائي إلى زيادة معنوية (P < 0.01) في نسبة نضج الذبيحة ووزن الأمعاء الدقيقة (الاثني عشر، والصائم، واللفائفي) مقارنةً بـ P0 وP1 وP2. أظهرت الطيور التي تغذت على مستخلص أنريديرا كورديفوليا النباتي انخفاضًا في مستوى الكوليسترول في البلازما، ولكن ارتفاعًا في مستوى الدهون الثلاثية في البلازما مقارنةً بالمعالجات الأخرى. بالإضافة إلى ذلك، أدى استخدام مزيج من مسحوق البروبيوتيك ومستخلص نبات أنريديرا كورديفوليا (P3) إلى تحسين ملحوظ في هضم المادة الجافة والمادة العضوية، ومستويات البيليروبين في البلازما (P<0.01). بينما لم تتأثر مستويات الألبومين والبروتين الكلي بالمعالجات. يُعزز مزيج مسحوق البروبيوتيك ومستخلص نبات أنريديرا كورديفوليا بشكل تآزري نسب الذبيحة وهضم العناصر الغذائية في دجاج التسمين.

الكلمات الدالة: دجاج التسمين، الهضم، خصائص الجهاز الهضمي، الحالة الأيضية، مستخلص نبات أنريديرا كورديفوليا، الأداء، البروبيوتيك.

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