

RESEARCH ARTICLE

Effect of Phytobiotic Carica Papaya Seed Extract on Growth Performance, Physiological Parameters, Gastrointestinal and Immune Organs of Broilers

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Abstract: Papaya (*Carica papaya*) seeds (PS) are known as a potential phytobiotic alternative to Antibiotic Growth Promotor (AGP) in poultry. Papaya seed extract (PSE) contains bioactive compounds such as carpine, chymopapain, and papain. Two hundred one-day-old Cobb strain broiler chicks were randomly allocated to five dietary treatments with four replicates per treatment, and each replicate consisted of ten birds. During the first 7 days, all birds were fed a basal diet for adaptation, after which the experimental diets containing 0% (ZE), 0.025% (LE), 0.05% (ME), 0.075% (HE), and 0.1% (VHE) PSE were provided for 28 days. Feed intake (FI), body weight gain (BWG), and feed conversion ratio (FCR) were recorded every week, while organ weights, intestinal morphometry, hematological values, and serum biochemical parameters were analyzed at the end of the trial. The results showed no significant differences ($p>0.05$) in growth performances, organ weights, or hematological indices among treatments. Serum albumin levels increased significantly ($p<0.05$) in birds in the LE group compared to the other groups. In conclusion, dietary inclusion with PSE in broiler diets can improve protein metabolism (albumin concentration) without adverse effects on growth, organ development, or blood health parameters up to 0.1% in the diet.

Keywords: Papaya seed extract, Performance, Metabolic serum, Hematology, Digestive organs

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Introduction

Antibiotics play an essential role in the poultry industry due to their cost-effectiveness in promoting growth. However, the use of antibiotics can lead to bacterial resistance and residues in animal products [1], posing the risk of cross-resistance and drug resistance in humans. The use of antibiotics as growth promoters in the poultry industry has been banned since 2006, necessitating the search for alternative substitutes [2]. Herbal plants and agricultural waste are increasingly being used as substitutes for antibiotics. Various vegetables, herbs, and seeds have been traditionally used as tonics [3].

Carica papaya Linn. (Caricaceae) is a tropical tree native to Central America and the West Indies, including regions around Mexico and Costa Rica, but is now widely cultivated in other tropical areas worldwide [4]. Among the components of the papaya (*Carica papaya*) plant, the seeds are particularly rich in oil, protein, and diverse bioactive compounds such as polyphenols, alkaloids, flavonoids, saponins, and proteolytic enzymes (papain and chymopapain) [5-6]. On a dry weight basis, papaya seeds contain up to 30% oil [7].

Previous studies from [8-9] reported that papaya seed extract contains alkaloids, flavonoids, saponins, tannins, anthraquinones, and anthocyanosides. Papaya seed extract (*Carica papaya* L.) contains organic acids, specifically monounsaturated fatty acids such as oleic acid (omega-9) and polyunsaturated fatty acids, including linoleic acid (omega-6) and linolenic acid (omega-3) [10]. Papaya seed extract has a high content of unsaturated fatty acids, such as oleic and palmitic acids [11]. The fatty acids in papaya seed oil include oleic acid (72-78%), palmitic acid (12-14%), stearic acid (4-5%), and linoleic acid (2.5-3.5%) [7]. [12] found that 0.5% and 1% papaya seed powder inclusion did not significantly affect feed efficiency or growth performance but positively influenced hematological parameters and significantly reduced serum lipid peroxidation. However, previous studies have focused mainly on the performance and hematological responses to papaya seed inclusion, with limited information on its effects on physiological organs and protein metabolism. Therefore, the present study aimed to evaluate different inclusion levels of papaya seed extract on growth performance, organ development, and serum biochemical indices in broiler chickens.

Materials and Methods

All procedures were approved by the Animal Ethics Committee of the Faculty of Animal Husbandry, Universitas Padjadjaran (Approval No: 0718070998). The study involved 200 Cobb strain broiler chicks as experimental subjects. Birds were allocated at n = 10 birds per replicate for each dietary treatment, following standard recommendations for broiler feeding trials commonly used in similar studies. The basal diet of the study, formulated to meet the nutrient requirements for broilers as recommended by NRC [13], and was supplemented with papaya seed extract at five inclusion levels: 0% (zero extract;ZE), 0.025% (low extract;LE), 0.05% (medium extract;ME), 0.075% (high extract;HE), and 0.1% (very high extract;VHE). The complete composition of the basal diet is shown in Table 1. Apart from the differing papaya seed extract inclusion levels, all treatment diets were identical.

Table 1: Composition of Basal Diet during the Trial

Ingredient	Composition (%)
Corn	62.8
Pollard	3.0
Soybean meal	8.0
Corn gluten meal	13.8
Rice bran	5.0
Meat bone meal	5.81
CaCO ₃	0.95
Methionine	0.07
Lysine	0.57
TOTAL	100

The chickens were raised in a litter system with good ventilation, and the coop was kept clean, with fumigation using formalin solution and lime applied to the walls and floor before housing the chickens. In the first week of rearing, feed was

provided ad libitum. From the second to the fourth week of age, feed was given twice daily, and drinking water was provided ad libitum. Feeders and drinkers were cleaned daily to prevent disease. Bird management followed standard commercial practices, including temperature control and routine vaccination.

Papaya seeds were obtained from the Gudeg Banda Bandung restaurant and fruit vendors in Jatinangor as waste material no longer in use. The seeds were collected in a detached state from the fruit. A total of 25 kg of wet seeds were obtained, washed thoroughly, and dried in an oven at 50°C for 48 hours. The seeds were then ground into flour and subjected to maceration for 48 hours with 70% ethanol. The solvent was evaporated to obtain the concentrated extract using a rotary evaporator. The resulting Papaya Seed Extract (PSE) was incorporated into the basal diet at the assigned inclusion levels for each dietary treatment.

Samples were taken at the end of the study (day 28) from one chicken per replicate, resulting in 20 samples in total. Sampling was based on the body weight of the chicken closest to the average weight in the pen. Before sampling, the chickens were fasted overnight. They were then weighed, blood samples were taken, slaughtered, and feathers were manually removed from the visceral organs, and the chicken carcass was recorded. Performance parameters recorded included FCR, weight gain, carcass percentage, abdominal fat, and consumption. The digestive tract was removed and divided into sections, starting from the entire digestive system, crop to colon. The digestive organs were cleaned of digesta, weighed to obtain the weight of each organ, and then converted to relative weight.

Blood was drawn from the wing vein of the chicken through the pectoralis vein, with 6 ml collected. Blood samples were stored in vacuum tubes pre-filled with Ethylene Diamine Tetra Acetyl Acid (EDTA), labeled according to the treatment, and placed in an ice thermos. Hematological parameters such as Red Blood Cells (RBC), White Blood Cells (WBC), hemoglobin (Hb), hematocrit, heterophil-lymphocyte ratio, and MID were measured. Blood serum was analyzed for blood glucose, triglycerides, cholesterol, total protein, urea, albumin, globulin, bilirubin, and creatinine.

The data obtained were analyzed using one-way analysis of variance (ANOVA). Significant differences between treatment means were determined using Duncan's multiple range test.

Results

Table 2: Weight of Digestive Tract, Bursa Fabricius, Heart, Liver, and Gallbladder

Percentage of Digestive Tract (%)	Treatment					P-value
	ZE	LE	ME	HE	VHE	
Weight	8.68	9.31	8.26	8.08	9.42	0.59
Crop	0.98	1.17	0.6	0.44	1.2	0.20
Proventriculus	0.58	0.65	0.54	0.54	0.61	0.74
Gizzard	2.32	2.79	2.39	2.06	2.62	0.83
Duodenum	1.18	1.35	1.17	1.41	1.32	0.80
Jejunum	1.49	1.47	1.52	1.35	1.50	0.97
Ileum	1.19	1.29	1.17	1.6	1.31	0.60
Caecum	0.57	0.53	0.58	0.54	0.60	0.23
Colon	0.34	0.28	0.27	0.19	0.29	0.26
Bursa Fabricius	0.09	0.10	0.10	0.09	0.12	0.40
Heart	0.67	0.67	0.68	0.73	0.87	0.61
Liver	3.18	3.05	3.30	3.00	3.23	0.96
Gallbladder	0.07	0.07	0.05	0.08	0.09	0.92

ZE = Zero Extract; LE = Low Extract; ME = Medium Extract; HE = High Extract; VHE = Very High Extract

Table 3: Growth Performance

Parameter	Treatment					P-value
	ZE	LE	ME	HE	VHE	
Feed Conversion Ratio (FCR)	1.72	1.73	1.71	1.74	1.84	0.93
Body Weight Gain (g/bird)	458.50	378.50	385.25	371.25	406.25	0.18
Carcass Percentage (%)	61.57	64.06	64.96	65.06	65.32	0.56
Abdominal Fat (%)	1.56	1.88	2.08	1.62	1.54	0.65

ZE = Zero Extract; LE = Low Extract; ME = Medium Extract; HE = High Extract; VHE = Very High Extract

Table 4: Serum Metabolites

Parameter	Treatment					P-value
	ZE	LE	ME	HE	VHE	
Glucose (mg/dL)	328.57	285.94	286.10	288	277.18	0.997
Triglycerides (mg/dL)	124.71	147.66	147.08	124.12	132.10	0.876
Cholesterol (mg/dL)	133.03	158.40	143.24	169.37	135.31	0.272
Total Protein (mg/dL)	4.181	4.711	3.96	3.96	3.57	0.384
Urea (mg/dL)	6.89	3.07	7.67	1.53	2.20	0.406
Albumin (mg/dL)	1.492 ^b	2.143 ^a	1.93 ^{ab}	1.91 ^b	1.71 ^b	0.012 [*]
Globulin (mg/dL)	2.689	2.568	1.93	2.05	1.89	0.466
Bilirubin (mg/dL)	13.15	11.58	11.65	11.96	11.72	0.167
Creatinine (mg/dL)	0.12	0.13	0.13	0.13	0.10	0.956

*Significant difference among treatments ($P < 0.05$). ^{ab}Different superscripts within a row indicate significant differences ($P < 0.05$). ZE = Zero Extract; LE = Low Extract; ME = Medium Extract; HE = High Extract; VHE = Very High Extract

Table 5: Hematology

Parameter	Treatment					P-value
	ZE	LE	ME	HE	VHE	
Erythrocytes (%)	2.25	2.43	2.5	2.52	2.45	0.562
Hemoglobin (g/dL)	10.65	11.2	11.93	11.8	11.65	0.763
Hematocrit	27	28.5	29.5	29.75	29	0.763
Leukocytes (mm ³)	82775	92050	80455	75700	91225	0.931
Heterophil/Lymphocyte Ratio	0.031	0.039	0.053	0.053	0.036	0.485
MID (%)	11	14	15	15.25	13.50	0.351

ZE = Zero Extract; LE = Low Extract; ME = Medium Extract; HE = High Extract; VHE = Very High Extract

The results of statistical analysis that giving no significant differences in the various treatments on organ weight were presented in Tables 2, FCR, weight gain, carcass percentage, and abdominal fat in Tables 3, and Erythrocytes, Hb, Hematocrit, Leukocytes, H/L Ratio, and MID presented in Tables 5. The results of albumin levels showed significant differences among the other parameters ($P < 0.05$) are presented in Tables 4, which showed LE had the highest albumin concentration by 2,143 mg/dL compared to other treatments.

Discussion

The final results of the study showed that the percentage weights of the digestive tract, crop, proventriculus, gizzard, intestine, bursa fabricius, heart, liver, and gallbladder did not differ significantly ($P > 0.05$; Table 2). However, the digestive organ weight in the VHE treatment tended to be higher, indicating that the administration of papaya seed extract as a herbal

plant contains compounds that can enhance nutrient absorption and digestion [12]. The antiparasitic, antimicrobial, and anthelmintic activity [14-16] of papaya seeds improves the gastrointestinal ecology in poultry, thereby enhancing health conditions and growth. This aligns with [17], who reported that papaya seed oil contains high levels of unsaturated fatty acids (particularly oleic and palmitic acids), which may contribute to improved nutrient absorption and gut health. [18] also reported the intestinal benefits of supplementing papaya even from its leaves. Including 3% papaya leaf in broiler diets improved gut integrity, indicating greater digestive efficiency and absorptive capacity. These effects were associated with increases in villus height and mucosal surface area, key determinants of nutrient uptake [19], as well as a healthier mucosa that likely limits colonization by pathogenic microorganisms [18]. This study highlights that adding PSE to the broiler diet does not have a diverse effect on the digestive tract; however, differences in processing (extract vs. powder/fermented meal), inclusion level, and dietary matrix may explain variation in responses across studies.

No significant differences were observed across treatments in FCR, weight gain, carcass percentage, and abdominal fat (Table 3). The administration of papaya seed extract up to the VHE level in the diet did not impact broiler performance. This finding is similar to [20], where the inclusion of papaya seed powder up to 1% in broilers yielded no notable effects, and [21], who demonstrated that fermented dried papaya seed flour can be incorporated up to 10% in broiler chicken diets without causing negative effects. Interestingly, the VHE group in this study exhibited the highest carcass percentage compared to other treatments, possibly due to better nutrient absorption, with higher relative intestinal weight compared to other treatments. This suggests that papaya seeds are rich in enzyme papain and bioactive compounds, including isothiocyanates and flavonoids, which possess potent antioxidant activity [22-23]. Papain is widely recognized for its proteolytic activity, which can improve protein digestion [24] and thereby potentially increase carcass weight. Antioxidants help mitigate oxidative stress in broilers by scavenging free radicals and reducing oxidative damage to cellular structures [25]. By alleviating oxidative stress, broilers may achieve better nutrient digestibility and metabolic efficiency. In addition, our findings also imply that anti-nutritional factors in PSE (e.g., tannins and phytic acid) did not exert measurable effects at the inclusion levels tested. Nevertheless, this study did not quantify the papain concentration, antioxidant levels, or anti-nutritional compounds in the PSE, which is a limitation.

Serum protein levels reflect the condition of an organism and the various changes that occur due to internal and external factors [26]. Blood serum albumin levels showed significant differences among the groups ($P < 0.05$; Table 4). Albumin levels in broilers fed papaya seed extract in the LE group were the highest among the treatment group at 2.143 g/dL. Our study indicated that adding 0.025% PSE to the basal diet may optimize liver function, which can trigger increased protein synthesis, leading to increased albumin levels. The liver is the primary site of albumin synthesis [27].

Erythrocytes, Hb, hematocrit, and H/L ratio showed no measurable variation (Table 5), indicating that feeding papaya seed extract was safe for the chickens' hematological parameters. Hematocrit is closely related to Hb, as hematocrit is approximately three times the Hb value [28]. The H/L ratio, a key indicator of stress and health status in chickens, remained consistently below 0.5 across all treatments (Table 5). Higher values of the H/L ratio are generally associated with stress and poor health, as an elevated ratio reflects an imbalance between heterophils and lymphocytes, often seen in stressful conditions. However, the stable H/L ratio observed in this study implies that papaya seed extract did not induce stress or health complications in the chickens. This supports the notion that papaya seed extract could potentially promote immune function by maintaining a healthy leukocyte balance without causing detrimental effects on overall health [4,10]. In conclusion, papaya seed extract has the potential to be used as a feed supplement that can enhance metabolic serum albumin levels, improve digestive organ performance, and support poultry health without adverse effects.

Conclusion

The inclusion of papaya seed extract in broiler diets increased serum albumin levels without negatively affecting growth performance, internal organ development, hematological indices, or serum biochemical parameters.

However, the present study was limited by the absence of molecular or microbiota analyses, making it difficult to determine the underlying mechanisms behind the albumin response. In addition, higher inclusion levels or longer feeding periods were not evaluated.

Despite these limitations, papaya seed extract shows potential as a natural phytobiotic ingredient for broiler production, particularly as an alternative to antibiotic growth promoters. Further studies investigating gut microbiota, digestibility, anti-

nutritional factors, and dose-response relationships are recommended to better define its optimal application in the poultry industry.

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Author's Contributions

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Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved. The experimental protocol was reviewed and approved by the Animal Ethics Committee of the Faculty of Animal Husbandry, Universitas Padjadjaran.

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