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Impact of Cassava Leaf Meal as a Rice Bran Substitute and Enzyme Supplementation on Lymphoid Organ Weight and Digestibility in Broiler Chickens

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ABSTRACT

Cassava leaves are a potential alternative feed ingredient due to their high levels of crude protein and energy. However, their utilization in poultry feed is constrained by factors such as cyanic acid, fiber content, and nutrient digestibility. This study aimed to investigate the impact of including cassava leaf meal (CLM) as a rice bran substitute and enzyme supplementation on lymphoid organ development and metabolizable energy parameters in broiler chickens. A 2x3 completely randomized factorial design was conducted using 48 Cobb-strain broilers unsexing at 35 days old. The treatments included different levels of CLM and enzyme (NSP and protease) supplementation at a dose of 250 g/ton of feed. The treatments consisted of R0E0: 0% CLM without enzyme, R0E1: 0% CLM with enzyme, R1E0: 1.5% CLM without enzyme, R1E1: 1.5% CLM with enzyme, R2E0: 3% CLM without enzyme, and R2E1: 3% CLM with enzyme. The variables assessed were lymphoid organ development (thymus, bursa Fabricius, and spleen) and metabolizable energy parameters (Apparent Metabolizable Energy (AME), True Metabolizable Energy (TME), Apparent Metabolizable Energy Corrected to Nitrogen (AMEn), and True Metabolizable Energy Corrected to Nitrogen (TMEn)). The data were subjected to analysis of variance (ANOVA) with post-hoc tests conducted for significant differences. Results indicated no interaction between CLM and enzymes in lymphoid organ development and energy metabolizable. The inclusion of CLM led to a reduction in AME and TME ($p<0.05$). However, enzyme supplementation significantly increased the relative weight of lymphoid organs (thymus, bursa Fabricius, spleen) and metabolizable energy parameters (AME, TME, AMEn, and TMEn) ($p<0.05$). Importantly, the inclusion of CLM up to a level of 3.0% did not negatively impact the health of broiler chickens. Furthermore, the addition of enzymes effectively mitigated the negative effects associated with CLM inclusion in the feed, suggesting their potential as a strategy to improve feed utilization in broiler production systems.

Keywords: Bursa Fabricius, Metabolizable, Local feed, Protein source, Spleen, Thymus

Introduction

In Indonesia, annual cassava production has reached approximately 21-24 million tons (BPS, 2015). Cassava leaves present a viable alternative source of feed ingredients due to their significant crude protein and energy content. According to a study conducted by Morgan and Choct (2016), cassava leaf meal can contain up to 23.78% crude protein and around 1,800 kcal/kg metabolizable energy. Furthermore, research by Salu and Paembonan (2010) demonstrated that including 1.5% to 3% cassava leaf meal as a feed supplement did not adversely affect the health of broiler chickens. This indicates that incorporating cassava leaves into poultry feed can offer a sustainable and cost-effective approach to provide the necessary protein and energy for optimal poultry growth and feed efficiency. Moreover, such integration can help reduce dependence on conventional feed ingredients like rice bran and soybean meal.

However, the utilization of cassava leaf meals as a feed ingredient in poultry feed faces limitations attributed to factors such as cyanic acid, fiber content, and nutrient digestibility. Oluwafemi and Omaku (2017) reported that cassava leaf meal is non-toxic up to a level of 20%; however, increasing its proportion in the feed leads to a decrease in body weight. Poultry, with their simple digestive system, have limited capacity to break down roughage, and cassava leaf meal, containing 17.69% crude fiber (Morgan and Choct, 2016), poses a challenge in this regard. Furthermore,

cassava leaves harbor antinutrients like hydrogen cyanide (HCN), which can be mitigated through various feed processing methods such as sun drying (Madalla *et al*., 2016), oven drying (Junior *et al*., 2019), and fermentation (Hermanto, 2018; Santos *et al*., 2019).

Cassava leaves are characterized by low digestibility due to their high fiber content. However, this limitation can be addressed by utilizing enzymes, which are protein-based catalysts that facilitate biochemical reactions (Uçak and Afreen, 2022). Enzyme supplementation in feed can break down complex compounds into simpler forms, leading to improved nutrient absorption. Notably, the addition of protease enzymes has been shown to enhance crude protein digestibility, trypsin activity, and intestinal morphology, including increased villi height, crypt depth, and villi-to-crypt ratio (Ding *et al*., 2016). Similarly, the inclusion of NSP enzymes in high-fiber feed materials can reduce intestinal viscosity, thereby improving overall digestibility (Simon, 1998).

The objective of this study was to assess the impact of incorporating cassava leaf meal as a substitute for rice bran in broiler chicken feed, coupled with enzyme supplementation, on lymphoid organ weight and metabolizable energy.

Materials and Methods

Feed preparation

The cassava leaves used in this study were of the Manggu variety, with red stems, jagged-shaped leaves, and aged 4-5 months. The leaves were separated from the stems, dried in a shaded area for 2-3 days, and then oven-dried for 24 hours at 60°C. After drying, the leaves were ground using a grinder.

Enzyme supplementation was conducted during the mixing process, in combination with micro-feed mixtures comprising amino acids and minerals. Two specific enzymes were utilized: NSP (Non-Starch Polysaccharide) enzyme, commercially known as Superzyme-CS, and protease enzyme, commercially known as Concentrase-P, both produced by Canadian Bio Systems. The dosage of each enzyme used was 250 g/ton, and the corresponding enzyme activity levels are presented in Table 1.

The rations were formulated according to the standard requirements for the pre-starter phase (1-7 days of age) based on SNI 8173.1, the starter phase (7-21 days of age) based on SNI 8173.2, and the finisher phase (21-35 days of age) based on SNI 8173.3 (BSN, 2015). The pre-starter phase ration served as the basal ration, while the treatment rations were given from the starter phase until the finisher phase.

The nutrients analyzed were dry matter, crude protein crude fat, crude fiber, calcium (Ca), phosphorus (P) based on AOAC (2005), and gross energy with the bomb calorimeter. The formula and nutrient content of the research rations is presented in Table 2.

Relative lymphoid organ weighting

Lymphoid organ weighing was performed on 35-day-old broiler chickens, unsexing, that were fed six different treatment rations, three with enzyme supplementation and three without (as listed in Table 2). Eight chickens from each treatment group were used to observe lymphoid organs, including the thymus, bursa Fabricius, and spleen. Additionally, the relative organ weight was calculated using the following formula:

Relative lymphoid organ weight (%) = Lymphoid organ weight (g) × 100 / Body weight (g).

Metabolizble energy measurement

Metabolizable energy was measured using a finisher ration applied to broiler chickens that were 35 days old, unsexing, and had previously been fed with the treatment feed (Table 2). A total of six treatments were used, each with four replications consisting of two chickens per replication. Digestibility was measured using metabolic cages that measured 50 cm x 30 cm x 56 cm in size, and an excreta collection tray was located beneath each cage.

Excreta was collected using the total collection method described by Tillman *et al*. (1984). The collection was carried out for three consecutive days at the end of the maintenance period in metabolic cages. For each replication, two samples were taken, and six samples were collected for endogenous measurement. Before excreta collection, the chickens were fasted for 24 hours but allowed free access to water. During the collection process, 0.01 N H2SO4 solution was sprayed on the excreta every three hours to prevent nitrogen evaporation. The excreta was then collected and stored in a freezer until analysis. The excreta was subsequently thawed, dried in an oven at 60°C, and ground using a mortar. The dried excreta was analyzed for its dry matter content, crude protein using the Kjeldahl method, and gross energy using a bomb calorimeter. The metabolizable energy calculations were based on the method described by Sibbald and Wolynetz (1985), using the following formula:

Apparent Metabolizable Energy (AME) = (EI - EE)/ FI, Apparent Metabolizable Energy corrected for nitrogen (AMEn) = (EI - (EE + NR * 8.22)) / FI, True Metabolizable Energy (TME) = (EI - (EE - EnE)) / FI, True Metabolizable Energy corrected for nitrogen (TMEn) = (EI - (EE - (EnE + NR * 8.22))) / FI

Where:

EI : Energy intake (kcal/kg)
EE : Energy excretion (kcal/kg)
FI : Feed intake (kg)
NR : Nitrogen retention (kg)
EnE : Endogenous excreta energy (kcal/kg).

Design and data analysis

The experimental design used in this study was a Completely Randomized Factorial Design. There were two treatment factors: Factor 1 involved

Table 1. Enzymes type and unit used in feed

Enzymes type	Activity (Unit g^{-1} enzyme)	Unit kg^{-1} feed
Protease enzyme	25,000	6,250
NSP (Non-Startch Polysaccharide) enzyme		
Xylanase	2,400	600
Glucanase	300	75
Invertase	1,400	350
Protease	2,400	600
Cellulase	1,000	250
Amylase	24,000	6,000
Mannanase	120	30
Pectinase	1,700	425

Table 2. Ingredient and nutritional content of research rations

Ingredient	Pre-starter (%)	Starter (%)			Finisher (%)		
		R0	R1	R2	R0	R1	R2
Corn	57.00	56.00	56.00	56.00	59.00	59.00	59.00
Rice bran	1.30	3.10	2.10	1.10	4.30	3.30	2.30
Soybean meal	26.00	26.50	25.50	24.50	21.00	20.00	19.00
Cassava leaf meal	0.00	0.00	1.50	3.00	0.00	1.50	3.00
Corn gluten meal	8.00	7.00	7.00	7.00	8.00	8.00	8.00
Crude palm oil	2.50	2.30	2.30	2.30	2.50	2.50	2.50
Fish meal	2.00	2.00	2.50	3.00	2.00	2.50	3.00
CaCO3	1.00	1.00	1.00	1.00	1.10	1.10	1.10
DCP	1.10	1.30	1.30	1.30	1.30	1.30	1.30
Premix	0.70	0.70	0.70	0.70	0.70	0.70	0.70
L-Lysin	0.10	0.05	0.05	0.05	0.05	0.05	0.05
DL-Methionine	0.10	0.05	0.05	0.05	0.05	0.05	0.05
Total	100	100	100	100	100	100	100
Nutrient content							
Dry matter (%)	85.91	85.77	85.88	85.88	85.70	85.82	85.93
Crude protein (%)	22.88	22.87	22.86	22.86	21.22	21.21	21.20
Crue fat (%)	5.22	5.02	5.08	5.08	5.39	5.45	5.52
Crude fiber (%)	2.36	2.75	2.91	2.91	2.83	2.90	3.14
ME (kal/g)	3,071	3,074	3,071	3,071	3,108	3,104	3,102
Calcium (%)	0.88	0.88	0.93	0.93	0.91	1.15	1.02
Phosporus (%)	0.51	0.51	0.53	0.53	0.50	0.52	0.53

R0: 0% cassava leaf meal, R1: 1.5% cassava leaf meal, R2: 3% cassava leaf meal.

cassava leaf meal addition with levels of 0% (R0), 1.5% (R1), and 3% (R3), and Factor 2 involved the addition of enzymes with two levels: without (E0) and with (E1) enzyme addition. All treatments had four replications. The variables observed in this study were the percentage of the relative weight of lymphoid organs, including the thymus, bursa Fabricius, and spleen, as well as metabolizable energy, including AME, AMEn, TME, and TMEn. The data were analyzed using analysis of variance (ANOVA). In cases where significant differences were found, a post-hoc test was conducted using Duncan's multiple range test, as implemented in SPSS 18 software (IBM Corp, 2009).

Results and Discussion

Relative weight of lymphoid organs

The lymphoid organs, including the thymus and bursa Fabricius, play a critical role in maintaining the body's defense system by producing lymphoid cells. The bursa Fabricius is considered the primary lymphoid organ, while the spleen is a secondary lymphoid organ (Wibawan and Soejoedono, 2013). Table 3 presents the relative weight of lymphoid organs in 35-day-old broiler chickens.

The experiment results revealed that there was no interaction between cassava leaf meal and enzyme addition on the relative weight of the lymphoid organs: thymus, bursa Fabricius, and spleen. However, enzyme addition increased the relative weight of the lymphoid organs (thymus, bursa Fabricius, and spleen) ($p<0.05$). This finding is in line with other research that showed enzyme supplementation could increase the level of immune defense cells (Montanhini *et al*., 2013; Cowieson *et al*., 2016). Additionally, enzymes were found to enhance the immune system by increasing the relative weight of the spleen, thymus, and bursa Fabricius (Attia *et al*., 2020).

The relative weight of the thymus and spleen fell within the normal range of 0.18% - 0.23% (Sturkie, 2000). However, the relative weight of the bursa Fabricius was lower than the expected minimum relative weight of 0.11%. A decrease in the relative weight of lymphoid organs indicates potential stress in poultry. This decrease is commonly attributed to heat stress, which triggers the release of corticosteroid hormones into the bloodstream to aid in metabolism (Lestari *et al*., 2020). Such weight reduction leads to a decrease in lymphocyte production, resulting in lower antibody levels in poultry (Kusnadi, 2009). Furthermore, environmental factors can also contribute to physiological changes. The temperature in the barn during the study ranged from 22.8 to 36.2°C, with a humidity level of 48-89%. The recommended temperature range for optimal broiler maintenance is 10-22°C (Nova, 2008). According to Fatmaningsih *et al*. (2016), chickens that feel uneasy in their surroundings may experience physiological changes. Elevated environmental temperatures can lead to heat accumulation within the body, resulting in heat

stress for the livestock. To address these concerns, various measures were implemented in this study. Curtains were opened and fans were used during high temperatures to enhance ventilation and cooling. Lamps were employed for heating during the night. Additionally, vitamin supplementation was administered through drinking water to support the overall health and well-being of the poultry.

Primary lymphoid organs serve as sites for the growth, development, embryogenesis, and maturation of immune system cells without the need for antigen exposure, while secondary lymphoid organs function as sites for lymphopoiesis and lymphocyte interaction with antigens, leading to maturation upon antigen exposure (Wibawan and Soejoedono, 2013). In poultry, the primary lymphoid organs consist of the Bursa Fabricius, which produces B cells, and the thymus, which produces T cells. The spleen serves as the secondary lymphoid organ.

Metabolizable energy

The study did not find an interaction between cassava leaf meal and enzymes on metabolizable energy values (Table 4). However, the addition of enzymes resulted in a difference ($p<0.05$) in EMS, EMM, EMSn, and EMMn. Feed supplemented with enzymes had a higher metabolizable energy value due to increased digestibility, which ultimately led to an increase in metabolizable energy value. Previous studies indicated that the addition of protease enzymes can increase metabolizable energy (Kalmendal and Tauson, 2012). Additionally, NSP enzymes have been shown to increase metabolizable energy (Romero *et al*., 2013). NSP enzymes can improve digestibility in both low and high-fiber feeds by reducing digesta viscosity, increasing protein concentration in the pancreas, and increasing chymotrypsin and lipase activity (Kalmendal and Tauson 2012).

According to Cho *et al*. (2020), the addition of protease enzymes can increase protein digestibility in low-protein feed. Protein can slow down the feed rate and increase nitrogen retention (Saraswati *et al.*, 2017). Nitrogen retention is a method to assess protein quality. Factors that affect protein digestibility are feed consumption, protein consumption, and protein quality (Fransisca *et al*., 2017). The addition of protease enzymes can also increase the true ileal digestibility of feed amino acids (Erdaw *et al*., 2019; Ghazi *et al*., 2002). The increase in protein digestibility with the addition of protease enzymes leads to optimal gluconeogenesis, resulting in an increase in metabolizable energy value (Lema-Pérez, 2021).

The inclusion of cassava leaf meal had a significant impact on EMS and EMM ($p<0.05$). Based on Duncan's post hoc test, a higher level of cassava leaf meal added resulted in a lower metabolizable energy value. This decrease in metabolizable

Table 3. The relative weight of lymphoid organs in 35-day-old broiler chickens

Parameter	Feed	Enzyme		Mean
		E0	E1	
Thymus (%)	RO	0.21 ± 0.03	0.24 ± 0.01	0.23 ± 0.02
	R1	0.21 ± 0.02	0.23 ± 0.01	0.22 ± 0.02
	R2	0.22 ± 0.02	0.23 ± 0.02	0.22 ± 0.02
	Mean	0.21 ± 0.02[b]	0.23 ± 0.01[a]	
Bursa Fabricius (%)	RO	0.09 ± 0.01	0.10 ± 0.01	0.09 ± 0.01
	R1	0.09 ± 0.01	0.10 ± 0.01	0.10 ± 0.01
	R2	0.09 ± 0.01	0.10 ± 0.01	0.09 ± 0.01
	Mean	0.09 ± 0.01[b]	0.10 ± 0.01[a]	
Spleen (%)	RO	0.19 ± 0.01	0.22 ± 0.01	0.21 ± 0.02
	R1	0.19 ± 0.01	0.21 ± 0.01	0.20 ± 0.01
	R2	0.18 ± 0.02	0.21 ± 0.01	0.20 ± 0.02
	Mean	0.19 ± 0.01[b]	0.22 ± 0.01[a]	

[a,b] Means in the same row without a common letter are different at $p<0.05$.
R0: cassava leaf meal 0%, R1: cassava leaf meal 1.5%, R2: cassava leaf meal 3%, E0: Without enzyme addition, E1: With enzyme addition.

Table 4. Metabolic energy in 35-day-old broiler chickens

Parameter	Feed	Enzyme		Mean
		E0	E1	
EMS (kkal/kg)	RO	3097.80 ± 32.07	3320.67 ± 35.92	3209.24 ± 131.64[a]
	R1	3074.52 ± 17.89	3273.46 ± 35.51	3173.99 ± 117.13[ab]
	R2	3036.82 ± 61.10	3167.97 ± 19.53	3102.40 ± 84.29[b]
	Mean	3069.72 ± 42.12[b]	3254.03 ± 74.00[a]	
EMM (kkal/kg)	RO	3412.69 ± 32.07	3610.62 ± 118.50	3511.66 ± 134.47[a]
	R1	3357.74 ± 8.04	3538.41 ± 11.71	3448.08 ± 104.63[ab]
	R2	3335.05 ± 33.17	3418.41 ± 24.49	3376.73 ± 53.69[b]
	Mean	3368.49 ± 41.40[b]	3522.48 ± 102.46[a]	
EMSn (kkal/kg)	RO	2987.61 ± 67.48	3144.01 ± 34.43	3065.81 ± 100.33
	R1	2946.48 ± 62.25	3116.25 ± 67.38	3031.36 ± 111.41
	R2	2919.23 ± 20.32	3080.34 ± 93.82	2999.29 ± 108.28
	Mean	2951.11 ± 52.12[b]	3113.53 ± 61.00[a]	
EMMn (kkal/kg)	RO	3302.50 ± 67.48	3433.97 ± 22.56	3368.23 ± 86.31
	R1	3219.71 ± 67.38	3406.20 ± 85.91	3312.95 ± 124.77
	R2	3182.46 ± 95.17	3390.78 ± 17.73	3286.62 ± 132.63
	Mean	3234.89 ± 81.55[b]	3410.31 ± 44.99[a]	

[a,b] Means in the same column for R1-R3 or same row for E0-E1 without a common letter are different at $p<0.05$.
R0: cassava leaf meal 0%, R1: cassava leaf meal 1.5%, R2: cassava leaf meal 3%, E0: Without enzyme addition, E1: With enzyme addition.

energy value could be attributed to the high crude fiber content present in cassava leaf meals. In this experiment, the control feed had a crude fiber content of 2.36%, while the feed containing CLM addition showed an increased crude fiber content ranging from 2.75% to 3.14% (Table 2). Although the crude fiber content in the feed remains below the recommended limit of 6% for crude fiber content, as stated in SNI 01-3930-2006, the observed increase in crude fiber from 2.36% to 3.14% is still significant enough to potentially impact the digestibility value. When forage-based feed is used, it can increase crude fiber, and this, in turn, can impact energy utilization (Ani *et al*., 2012). However, the use of enzymes in cassava leaf meals can aid in breaking down and digesting the feed, resulting in an increase in metabolizable energy. This finding is consistent with a previous report by Ridla *et al*. (2019) which stated that an increase in crude fiber content in the feed leads to a decrease in metabolizable energy level and that this can be mitigated by the addition of enzymes.

Conclusions

The study found that broiler feed containing cassava leaf meal with added enzymes increased the metabolizable energy in 35-day-old broiler chickens without compromising animal health. The use of enzymes in feed improved digestibility and reduced the harmful effects of adding cassava leaf meal to the diet.

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