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The effectiveness of cassava leaf meal (*Manihot esculenta* Crantz) in feed with enzymes supplementation on Broiler digestive organs

Ririn Angriani[1], Widya Hermana[2] and Muhammad Ridla*[2]

[1]Graduate Study Program of Nutrition and Feed Science, IPB University, Bogor 16680, Indonesia
[2]Department of Nutrition and Feed Technology, Faculty of Animal Science, IPB University, Bogor 16680, Indonesia

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ABSTRACT: This research aimed to evaluate the effect of cassava leaf meal with enzyme supplementation in feed on the digestive organs of the broiler. The research used a completely randomized factorial design on 48 Cobb-strain broilers distributed into two observation factors and four replications (consisting of 2 samples). The observation factor is factor 1 is the addition of cassava leaf meal (0%; 1.5%; and 3.0%), and the second factor is the addition of non-starch polysaccharides and protease enzymes with each dose of 250 g/ton feed (with and without enzymes). The variables analyzed were the relative weight of the digestive organs, the relative weight of the parts of the small intestine, and the relative length of the parts of the small intestine. The data obtained were analyzed by analysis of variance (ANOVA), and if the results indicated significantly different, then the posthoc test was conducted. The results showed that the addition of cassava leaf meal could increase the relative weight of the gizzard significantly ($p<0.05$). The addition of enzymes can significantly decrease the relative weight of the gizzard, small intestine, pancreas, ileum, and the relative length of the duodenum and ileum ($p<0.05$). The use of cassava leaf meal in the feed up to 3.0% does not interfere with digestive organs. There was no interaction between cassava leaf meal and enzymes on size response of digestibility broiler organ. The addition of enzymes can offset the detrimental effects caused by the use of cassava leaf meals in feed.

Keywords: Broiler; Cassava leaf meal; Digestive organ; Enzyme; Local feedstuffs

*Corresponding Author: hmridla@apps.ipb.ac.id

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INTRODUCTION

Protein is one of the nutrients needed for broiler growth and feed efficiency. The primary source of protein in poultry feed is soybean meal with high protein and digestibility. The total soybean import was 2.67 million tons in 2019 (BPS, 2019). One of the alternatives to overcome the dependence on imports was utilizing a local feedstuff protein source. Cassava is a local plant with production in Indonesia that reached 19.3 million tons in 2018 (Kementan, 2018). The part of cassava is the tuber, skin of tuber, stem, and leaf. 10-40% of cassava plants consist of leaves. Cassava leaves contain high crude protein. Crude protein content in cassava leaf meal reaches 23.78% (Morgan and Choct, 2016). Salu and Paembonan (2010) reported that adding cassava leaf meal levels of 1.5% and 3.0% could increase weight gain with a lower feed conversion ratio. The nutritional content of cassava leaves varies with each age (Wobeto *et al.,* 2006). The older the leaf, the protein, and amino acid levels will decrease, while the crude fiber content will increase.

Cassava leaves have a constrain high crude fiber and have antinutrients. The crude fiber content in cassava leaf meal reaches 17.69% (Morgan and Choct, 2016). The crude fiber in poultry helps intestinal peristaltic movements, prevent clumping of feed on the cecum, accelerates the digestion rate, and triggers the development of digestive organs. However, chickens have limitations in the breakdown of crude fiber due to the simple anatomy of the digestive tract of chickens. Cassava leaves have antinutrients such as cyanide acid (HCN). HCN in fresh cassava leaves can reach 560.9 mg/kg (Junior *et al.,* 2019). HCN tolerance limits in chickens range from 0.5-3 mg/kg body weight (Hidayat, 2009). Reducing the HCN levels through feed processing has been done in several studies. According to Fasuyi (2005), processing methods to reduce level HCN were sun-drying, oven-drying, steaming, shredding, and steeping. 60% of the HCN content is reduced the HCN content in cassava leaves with sun drying (Madalla *et al.*, 2016). Cassava leaves withered in the shade can also reduce the HCN content by 58% (Hang and Preston, 2005). Drying with an oven can also reduce 74.1-92.2% HCN (Junior *et al.,* 2019). In addition, the HCN content can also decrease in the fermentation process to 99.74% (Hermanto, 2018). However, processing methods can also reduce the nutritional content value (Santos *et al*., 2019).

High crude fiber and antinutrients cause cassava leaves to have low digestibility. Oluwafemi and Omaku (2017) stated a decrease in body weight and an increase in the level of cassava leaf meal in the feed. Giving enzymes can overcome low digestibility. Non-starch polysaccharides (NSP) enzymes can overcome the problem of high fiber broiler feed (Makhdum *et al*., 2013). The utilization of protein of cassava leaf meal can also be the addition of protease enzymes. The addition of enzymes can increase the digestibility of nutrients in feed. Protease enzymes can increase crude protein digestibility, trypsin activity, and intestinal morphology (Ding *et al.,* 2016). This research aimed to evaluate the effect of adding cassava leaf meal in feed with enzyme supplementation on the broiler digestive organs. Finally, it can be used as a reference for utilizing cassava leaf meal in feed.

MATERIALS AND METHODS

Animal

This research used broiler strains of the Cobb since Day Old Chick (DOC) was raised. This research used 48 broilers distributed into six treatments and four replications (each replication consisted of 2 samples).

Cassava Leaf Meal (CLM)

The cassava leaves used were manggu cassava variety with the red leaf-stem cassava, finger-shaped leaves, and aged 4-5 months old. 15-20 leaves on the top of leaf plants taken cassava leaves. The stems were separated from cassava leaves.

Furthermore, the fresh leaves were dried in the shade for 2-3 days, then put into the oven for 24 hours at 60 ^{o}C. The chemical content of cassava leaf meal can be seen in Table 1.

Table 1. Chemical content of cassava leaf meal (CLM)

Chemical	Content
Dry matter (%)	94.01
Ash (%)	6.45
Crude protein (%)	23.00
Crude fiber (%)	13.11
Crude fat (%)	1.37
Gross energy (cal/g)	4090
Calcium (%)	0.79
Phosphor (%)	0.38
Cyanide acid (mg/kg)	79.29

Enzymes

The enzyme used was NSP (Non-Starch Polysaccharide) with Superzyme-CS brand and protease enzyme with Concentrase-P brand extracted from *Bacillus lichenciformis*. The enzyme each dose used was 250 g/ton feed. The level of enzyme activity used can be seen in Table 2.

Table 2. Level of enzyme activity

Enzyme	Enzyme activity in product (unit/g)	Enzyme activity in feed (unit/kg)
Protease	25000	6250
Non-Starch Polysaccharide (NSP)		
Xylanase	2400	600
Glucanase	300	75
Invertase	1400	350
Protease	2400	600
Cellulase	1000	250
Amylase	24000	6000
Mannanase	120	30
Pectinase	1700	425

Source: Canadian Bio-System

Diet

Ration formulation adapted to nutrient requirements of broilers at pre-starter phase (age 1-7 days) based on SNI 8173.1:2015, starter (age 7-21 days) based on SNI 8173.2:2015, and finisher (age 21-35 days) based on SNI 8173.3:2015 (BSN, 2015). The ration given to the pre-starter phase chickens was the basal ration—furthermore, the starter phase until the finisher given the treatment ratio. The nutrient content that was analyzed was the dry matter and crude fiber based on the method of SNI 01-2891-1992; crude protein, crude fat, and calcium (Ca) based on the method of AOAC, 2005; phosphor (P) based on the gravimetry method; and gross energy by bomb calorimeter. The formulation and nutrient value of the experimental ratio are present in Table 3.

Table 3. Formulation and nutrients value of the experimental ration

Feedstuffs	Pre starter (%)	Starter (%)			Finisher (%)		
	Basal	K_0	K_1	K_2	K_0	K_1	K_2
Maize	54.00	56.00	56.00	56.00	59.00	59.00	59.00
Rice bran	4.30	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
Calcium carbonate	1.00	1.00	1.00	1.00	1.10	1.10	1.10
Dicalcium phosphate	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-Metionin	0.10	0.05	0.05	0.05	0.05	0.05	0.05
Total	100	100	100	100	100	100	100
Nutrients value							
Dry matter (%)	85.91	85.77	85.88	85.88	85.70	85.82	85.93
Crude protein (%)	22.33	22.87	22.86	22.86	21.22	21.21	21.20
Crude fat (%)	5.22	5.02	5.08	5.08	5.39	5.45	5.52
Crude fiber (%)	2.86	2.75	2.91	2.91	2.83	2.90	3.14
Gross energy (cal/g)	3070.8	3074.2	3070.9	3070.9	3108.3	3104.9	3101.6
Calcium (%)	0.88	0.88	0.93	0.93	0.91	1.15	1.02
Phosphor (%)	0.51	0.51	0.53	0.53	0.50	0.52	0.53

K0: ration without CLM; K1: ration with 1.5% CLM; K2: ration with 3.0% CLM

Digestive organ measurement

Eight chickens 35 days old from each treatment were slaughtered, then the feathers were removed, and the organs were separated. Weighed the organs one by one, and the length of the intestine was measured using a ruler.

The organs observed included the proventriculus, gizzard, small intestine, pancreas, liver, and the small intestine consisting of the duodenum, jejunum, and ileum. The gizzard and small intestine observed were a net weight that the contents had been cleaned. The length of the parts of the small intestine is divided based on the division of the small intestine, namely the duodenum (the U-shaped or surrounds the pancreas), jejunum (from the end of the duodenum to Meckel's diverticulum), and ileum (from Meckel's diverticulum to the beginning of the cecum branch), next, calculated using the following formula for the relative weight of the organs and the relative length of the intestine.

$$\text{Relative weight of organs (\%)} = \frac{\text{Weight of organs (g)}}{\text{Body weight (g)}} \times 100\%$$

$$\text{Relative length of intestine (cm/100g)} = \frac{\text{Intestine length (cm)}}{\text{Body weight (g)}} \times 100$$

Experimental Design and Data Analysis

This research used a completely randomized factorial design into two observation factors distributed in this study. The observation factor is factor 1, the addition of cassava leaf meal (0%; 1.5%; and 3.0%), and the second factor is the addition of non-starch polysaccharides and protease enzymes with each dose of 250 g/ton of feed (with and without enzymes). There were six treatments with four replications, namely P1: K0, P2: K0 + enzyme, P3: K1, P4: K1 + enzyme, P5: K2, and P6: K2 + enzyme.

The variables analyzed were the relative weight of the digestive organs, the relative weight of the parts of the small intestine, and the relative length of the parts of the small intestine. The data obtained were analyzed by analysis of variance (ANOVA). If the results indicated significantly different at significance level 0,05, then the post-hoc Duncan means range test (DMRT) was performed.

RESULT AND DISCUSSION

Digestive Organ

The digestive system functions in the absorption of feed nutrients to meet the needs of life. Three digestive processes occur in poultry. Mechanical digestion occurs in the gizzard, enzymatic digestion occurs in the small intestine, and fermentative digestion occurs in the cecum. The relative weight of digestive organs in 35-day-old broilers is present in Table 4.

There was no interaction between cassava leaf meal and enzymes on the digestive organs, but adding enzymes can significantly decrease the relative weights of the gizzard, small intestine, and pancreas ($p<0.05$). Larger organ sizes are an indication that the organ is working harder (Widjaja, 2012). Organs with enzymes had a lower average relative weight than organs without enzymes. This is because enzymes can reduce the adverse effects of antinutrients and increase nutrient digestibility. The small intestine functions in the enzymatic absorption of feed nutrients. Amylase, protease, and lipase enzymes secreted by the pancreas function in breakdown feed nutrients into simple forms to be absorbed by the body.

The amylase enzyme functions to help the breakdown of starch, lipase help the breakdown of fat, and trypsin help the breakdown of protein. Previous studies have shown that protease supplementation can increase crude protein digestibility and trypsin activity in the pancreas and small intestine (Ding *et al.*, 2016; Yuan *et al.*, 2017; Erdaw *et al.*, 2019). The increase in digestibility and trypsin activity led to a decrease in the relative weights of the pancreas (Yuan *et al.*, 2008; Erdaw *et al.*, 2019) and small intestine (Yuan *et al.*, 2008; Erdaw *et al.*, 2017). NSP enzyme can also increase amylase activity in the digestive tract (Yuan *et al.*, 2008). NSP enzyme was able to reduce the relative weight of the gizzard (Essien *et al.*, 2018).

The addition of cassava leaf meal can increase the relative weight of the gizzard significantly ($p<0.05$). The research is in line with (Diarra and Annand, 2020; Widjaja, 2012) that cassava leaf meal can increase the relative weight of the gizzard. Besides that, cassava leaf meal can increase the relative weight of the proventriculus (Diarra and Annand, 2020) and heart (Widjaja, 2012).

This was due to the high crude fiber content in cassava leaf meals. Gizzard consists of muscle fibers that function to reduce food mechanically. Factors that affect the size of the gizzard include livestock condition, size of livestock, type of feed, the volume of feed consumed, and form of feed. The crude fiber of feed was the main factor that can affect the weight of the gizzard (Hetland *et al.*, 2005). Has *et al.*, (2014) stated that an increase in crude fiber can affect the weight of the digestive tract, especially in the gizzard, small intestine, and caecum. Crude fiber can affect organ weight due to an increase in organ performance in digesting crude fiber (Dianti, 2012).

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Table 4. The relative weight of digestive organs on a 35-day-old broiler

Parameter	Feed	Enzymes		Average
		Without	With	
Proventriculus (%)	K0	0.61 ± 0.02	0.59 ± 0.01	0.60 ± 0.02
	K1	0.59 ± 0.06	0.57 ± 0.06	0.58 ± 0.06
	K2	0.56 ± 0.04	0.58 ± 0.04	0.57 ± 0.04
Average		0.59 ± 0.04	0.58 ± 0.04	
Gizzard (%)	K0	2.12 ± 0.04	1.80 ± 0.07	1.96 ± 0.18[b]
	K1	2.20 ± 0.02	1.86 ± 0.11	2.03 ± 0.20[ab]
	K2	2.30 ± 0.07	1.93 ± 0.19	2.12 ± 0.24[a]
Average		2.21 ± 0.09[a]	1.86 ± 0.13[b]	
Small intestine (%)	K0	4.32 ± 0.43	4.12 ± 0.22	4.22 ± 0.34
	K1	4.47 ± 0.41	4.18 ± 0.16	4.32 ± 0.33
	K2	4.53 ± 0.19	4.24 ± 0.21	4.38 ± 0.24
Average		4.44 ± 0.34[a]	4.18 ± 0.19[b]	
Pancreas (%)	K0	0.32 ± 0.02	0.26 ± 0.01	0.29 ± 0.03
	K1	0.33 ± 0.04	0.28 ± 0.03	0.31 ± 0.04
	K2	0.33 ± 0.01	0.30 ± 0.01	0.31 ± 0.02
Average		0.33 ± 0.03[a]	0.28 ± 0.03[b]	
Liver (%)	K0	2.63 ± 0.13	2.64 ± 0.16	2.64 ± 0.14
	K1	2.71 ± 0.20	2.71 ± 0.40	2.71 ± 0.13
	K2	2.71 ± 0.10	2.79 ± 0.10	2.75 ± 0.10
Rataan		2.67 ± 0.14	2.72 ± 0.12	

Numbers followed with a different superscript in the same column-subgroup showed significantly different ($p<0.05$).
K0: ration without CLM; K1: ration with 1.5% CLM; K2: ration with 3.0% CLM

Small Intestine

The small intestine is the site of absorption of feed nutrients. The small intestine is divided into the duodenum, jejunum, and ileum. The relative weight and length of the parts of the small intestine in 35-day-old broilers are present in Table 5.

The addition of enzymes can significantly decrease the relative weight and length of the duodenum, jejunum, and ileum ($p>0.05$), but there was no interaction between cassava leaf meal and enzymes on the relative weight and length of the duodenum, jejunum, and ileum. Organs with enzymes had a lower average relative weight and length than organs without enzymes. The small intestine is the main site of enzymatic digestion. The duodenum functions to absorb nutrients, the jejunum absorbs other nutrients that the previous process has not absorbed, and ileum is a place of absorption of water and minerals. Feed size, hardness, solubility, and enzyme activity influence digestibility (Yang *et al.*, 2013). The decreased weight and length of the small intestine is a form of response to the addition of enzymes due to the work of the small intestine that does not try too hard to absorb feed nutrients. According to Wang *et al.*, (2016), a longer small intestine indicates a greater area of digestion and absorption of nutrients. The addition of enzymes can reduce the relative weight of the small intestine (Yuan *et al.*, 2008; Erdaw *et al*., 2017).

Enzyme supplementation can reduce the adverse effects of antinutrients, thereby improving duodenal performance (Ding *et al*., 2016). In addition, the addition of exogenous enzymes in the feed can improve the intestinal morphology of broiler chickens (Yuan *et al.,* 2008). Supplementation of protease enzymes in broiler chicken feed can improve physiological functions by secreting functional enzymes and changing the intestinal structure to increase nutrient absorption (Erdaw *et al*., 2017). Previous

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studies have stated that adding proteases to feed can increase the digestibility of crude protein and amino acids (Erdaw *et al.*, 2019; Cho *et al.*, 2020; Ghazi *et al.*, 2002). The addition of NSP can increase nutrient digestibility (Toghyani *et al.*, 2017).

Table 5. Relative weight and length of parts of the small intestine on a 35-day-old broiler

Parameter	Feed	Enzymes		Average
		With	Without	
Duodenum weight (%)	K0	1.13 ± 0.07	1.03 ± 0.06	1.08 ± 0.08
	K1	1.14 ± 0.08	1.09 ± 0.09	1.11 ± 0.08
	K2	1.15 ± 0.10	1.12 ± 0.09	1.13 ± 0.09
Average		1.13 ± 0.08	1.08 ± 0.08	
Jejunum weight (%)	K0	1.80 ± 0.06	1.74 ± 0.06	1.77 ± 0.06
	K1	1.83 ± 0.06	1.80 ± 0.09	1.81 ± 0.07
	K2	1.87 ± 0.05	1.82 ± 0.12	1.85 ± 0.09
Average		1.83 ± 0.06	1.79 ± 0.09	
Ileum weight (%)	K0	1.68 ± 0.05	1.53 ± 0.05	1.60 ± 0.09
	K1	1.71 ± 0.13	1.61 ± 0.12	1.66 ± 0.13
	K2	1.78 ± 0.07	1.65 ± 0.09	1.72 ± 0.11
Average		1.72 ± 0.10^{a}	1.60 ± 0.10^{b}	
Duodenum length (cm/100 g)	K0	3.85 ± 0.19	3.64 ± 0.10	3.74 ± 0.18
	K1	3.90 ± 0.19	3.70 ± 0.12	3.80 ± 0.18
	K2	3.85 ± 0.12	3.80 ± 0.12	3.82 ± 0.11
Average		3.87 ± 0.16^{a}	3.71 ± 0.12^{b}	
Jejunum length (cm/100 g)	K0	9.00 ± 0.42	8.77 ± 0.13	8.88 ± 0.31
	K1	9.06 ± 0.32	8.80 ± 0.53	8.93 ± 0.42
	K2	9.15 ± 0.48	8.88 ± 0.35	9.02 ± 0.41
Average		9.07 ± 0.38	8.82 ± 0.34	
Ileum length (cm/100 g)	K0	9.14 ± 0.14	8.82 ± 0.10	8.98 ± 0.21
	K1	9.08 ± 0.06	8.86 ± 0.09	8.97 ± 0.14
	K2	9.11 ± 0.47	8.90 ± 0.31	9.01 ± 0.37
Average		9.11 ± 0.24^{a}	8.86 ± 0.18^{b}	

Numbers followed with a different superscript in the same column-subgroup showed significantly different ($p<0.05$).
K0: ration without CLM; K1: ration with 1.5% CLM; K2: ration with 3.0% CLM

The addition of cassava leaf meal had no significance on the relative weight and length of the duodenum, jejunum, and ileum. The higher the level of cassava leaf meal, so higher the relative weight and length of the small intestine produced. This is due to the high crude fiber content in cassava leaf meals.

High crude fiber can increase the length of the small intestine and large intestine (Yakabu *et al.,* 2017). The addition of enzymes can help break down and digest feed in cassava leaf meal which contains antinutrients (Oluwafemi and Omaku, 2017).

CONCLUSIONS

Feed with cassava leaf meal, and additional enzymes can be applied in the field. Broiler feed with cassava leaf meal can increase the relative weight of the gizzard, but the use of cassava leaf meal in the feed up to a level of 3.0% does not interfere with digestive organs. The addition of NSP and protease enzymes can decrease the relative weight of the gizzard, proventriculus, small intestine, and the length of the duodenum and ileum because the addition of enzymes can offset the detrimental effects caused by the use of cassava leaf meal in feed. Further research can be done, such as testing crude fiber and anti-nutritional content in cassava leaf meals to determine the limiting factors in the use of cassava leaf meal in broiler chickens so that the user can be more optimal.

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