ISSN: 0852-3681
E-ISSN: 2443-0765

Jurnal Ilmu-Ilmu Peternakan 31(3): 201 – 210
Available online at http://jiip.ub.ac.id

Utilization of jack bean seed (*Canavalia ensiformis*) with treatments of protease enzyme fed to broiler in starter period

Brahmadhita Pratama Mahardhika*[1], Muhammad Ridla[1)*], Rita Mutia[1], Iyan Yuliana[1] and Miftakhun Naja[1]

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

Submitted: 10 July 2020, Accepted: 25 August 2021

ABSTRACT: The achievement of this study was to evaluate the use of jack bean seed and protease enzymes in different protein levels in the Broiler ration on performance and metabolizable energy. Two hundred and forty broilers were distributed into 3 object factors and 3 replications. The first factor was the difference of the ingredients (soybean meal-based basal ration and jack bean seed-contained rations), the second factor was the level of protein (22% and 19.5%) and the third factor was the enzyme treatment (without enzymes and with the inclusion of the proteases). A total of 2 birds from each treatment were assigned as a sample for metabolizable energy assay. The experimental design used was a factorial completely randomized design (FCRD). The data were analyzed using Analysis of Variance (ANOVA). The results showed that adding jack bean seed into the Broiler starter's ration significantly (P <0.01) decreased the body weight gain (BWG) but it could be improved with the inclusion of protease enzyme. The metabolizable energy of the ration containing jack bean seed was significantly higher (P<0.01) than in the basal ration. The use of a low protein level ration (19.5%) significantly (P <0.01) showed the decrease in BWG, decrease in true metabolizable energy, and true corrected-nitrogen metabolizable energy but could be improved with the addition of protease enzyme. The use of the protease enzyme could improve the performance of broiler chickens fed with a low protein ration that contained Jack bean seed (*Canavalia ensiformis*).

Keywords: Metabolizable energy; Performance; Protease enzyme

*Corresponding Author: hmridla@yahoo.com

INTRODUCTION

Broiler Chicken is one of the livestock-producing commodities of meat that has a fairly good nutritional value, especially its protein content. The advantages of the meat are its soft texture and delicious taste. Broiler chickens can be caged and raised in a short period, which is about 35 days. High broiler meat production must be balanced with a good quality of the ration.

A good ration is always sufficient for livestock, has good nutritional value and digestibility, is available throughout the year, and has a low price. The ration of the broiler chicken in Indonesia is based on corn as an energy source and soybean meal as a source of protein. Soybean meal is a by-product of the process of producing soybean oil. Indonesia is not a soybean oil-producing country, so the by-product in the form of soybean meal still has to be imported and in 2016, Indonesia imported soybean meal up to 3 million tons (KEMENDAG 2017).

One of the local ingredients that are mostly found in Indonesia as a source of protein is jack bean seeds (Canavalia ensiformis). Jack bean seeds had a crude protein content of 22.8% - 35.3% (Sridhar and Shena, 2006). Jack bean seeds were found on the Indonesian island of Java. The area of land planted with jack bean seeds in Indonesia was 1590 ha with a production of 5 tons per hectare per year (Kasno 2016). The price of soybean meal protein is in the range of IDR 205,510.00 per kg of protein, while the price of protein from Jack bean seed is only IDR 127,460.00 per kg of protein. The disadvantage of Jack bean seeds compared to soybean meal is its high amount of antinutrient in the form of trypsin inhibitor. According to Lukiwati and Prawiradiputra (2014), the trypsin inhibitor content is 8.9 units per gram. In addition to trypsin inhibitors, other toxin substances in jack bean seeds are kholin, hydrozianine acid, troginelin and antichimotrypsin, phenols, isoflavones, niacin, saponins (Primawestri and Rustanti, 2014). Trypsin inhibitor is a type of protein antinutrient that inhibits the action of the trypsin enzyme in the body so that protein absorption will be inhibited (Liu, 1999). Some procedures that can be done to reduce trypsin inhibitor content are soaking and heating. The heating processes are including boiling, steaming, or autoclaving (Hilyati and Benti, 1991).

Digestibility is part of the consideration to determine the quality of materials to be a source of feed ingredient. One of the efforts to improve digestibility is the utilization of enzymes. Enzymes are biocatalysts in accelerating a chemical reaction in living organisms. Enzymes are also a protein that can be denatured when they are at high temperatures and other extreme conditions (Suhartono, 2000). The enzyme that is used to improve protein digestibility is the protease enzyme. Protease enzymes can catalyze the breakdown of peptide bonds in polypeptides and proteins through hydrolysis reactions into simpler molecules such as short-chain peptides and amino acids (Naiola and Widyastuti, 2002).

Feed ingredients with high crude protein content do not necessarily have good quality in terms of digestibility. An ingredient that has high protein content but cannot be digested properly will be useless. Efforts can be done to increase the digestibility of crude protein was adding protease enzymes to the ration. Protease enzyme inclusion on ration could increase protein digestibility so that metabolic energy could be improved in a state of energy deficiency and the gluconeogenesis could work properly. Gluconeogenesis is the entire mechanism and the process of converting all non-carbohydrate compounds into glucose (Sari, 2009). These efforts are expected to be able to optimize the use of a lower protein ration than its requirement to be as same as the use of a high protein ration. In addition, the inclusion of the protease enzyme was expected to improve the digestibility of the poor-quality ingredients. This study aimed to evaluate the use of jack bean seed and the inclusion of protease enzymes in the ration with different protein levels fed to broilers in the starter period.

MATERIALS AND METHODS

This research was conducted in 2 stages. The first stage was to measure the performance of broiler chickens raised from Day Old Chick (DOC) until the starter phase (21 days). After that, the second stage was to measure the metabolizable energy in broiler chickens.

Birds and Housing

The birds used in the first stage of the study were 240 male broilers Lohmann MB 202 strain and distributed into 3 observation factors and 3 replications. The factors were ingredients (soybean meal-based basal ration and rations containing jack bean seeds), protein levels (22% and 19.5%), and enzyme treatment (with and without the addition of protease enzyme). Each replication consisted of 10 chickens. In the second stage, broiler chickens were assigned for performance measurement, a total of 2 chicken samples for each replication in each treatment. In the measurement of metabolizable energy, fifty-six broilers were assigned and 48 birds were for the total collection of excreta and 8 birds were for the endogenous losses assay.

The cage used in the first phase of the study was a 1 m x 1 m colony cage. The cage used for the second stage was a 50 cm x 30 cm x 56 cm metabolic cage.

Rations

In the pre-starter phase using commercial ration and in the starter phase using ration formulated based on Leeson and Summer (2005)'s recommendations. Each formulated ration was added with enzymes and without enzymes. The enzyme used was protease enzyme-containing 12,500 hemoglobin units (HUT) / g as much as 0.5 g / kg of ration. The composition and nutrients of the starter phase broiler chicken ration were presented in Table 1.

Enzymes

The enzyme used was a commercial exogenous protease enzyme in powder form. The protease enzyme was extracted from *Bacillus licheniciform*is under the brand name concentrase-P (Canadian Biosystems, 2015). The composition of the protease enzyme used was wheat flour, lime, and silicon dioxide. The concentration of the enzyme used was 12500 HUT / g (hemoglobin unit per gram).

Procedure

Analysis of Trypsin Inhibitor Concentration

Jack bean seed meal was heated using an autoclave at 26 ° C at 1 atm for 25 minutes to reduce the content of trypsin inhibitor. The analysis of trypsin inhibitor concentration was carried by the Soetrisno and Suryana (1991) method by analyzing the wavelengths using a spectrophotometer. the data of the analysis of trypsin inhibitor concentration were presented in Table 2.

According to the results of the analysis, it was decided to use autoclave treatment for jack bean seed meal. The autoclave treatment applied the principle of heating and pressure. The autoclave technique was able to reduce the concentration of trypsin inhibitor in Jack bean seed meal (*Canavalia ensiformis*).

Maintenance and Performance measurement

Broiler chickens were caged from DOC to the starter phase (21 days). Every week, the chickens were measured for body weight, body weight gain, and feed intake. Bodyweight was measured using digital scales. Bodyweight gain was measured by calculating the difference between initial body weight and final body weight. Feed intake was measured by calculating the difference between the feed provided and the feed residue. On the first day of the experimental period, DOC was given 10% brown sugar-contained water and fed with commercial vitamin with brand Vita Stress.

DOI: 10.21776/ub.jiip.2021.031.03.03

Table 1. Feed composition and nutrient content of Broiler chicken ration starter phase

Ingredients (%)	A basal ration with 22% CP	A basal ration with 19.5% CP	Jack bean seed-based ration with 22% CP	Jack bean seed-based ration with 19.5% CP
Yellow Corn	50.50	55.00	48.50	52.85
Rice bran	10.40	10.40	8.50	9.00
Crude Palm Oil	2.20	2.40	2.20	2.20
Corn Gluten Meal	8.00	3.20	7.00	3.00
Soybean meal	25.90	25.90	23.70	22.80
Jack bean Seed meal	0.00	0.00	7.00	7.00
Premix	0.50	0.60	0.50	0.50
calcium carbonat	0.60	0.30	0.40	0.40
Dicalcium Phosphate	1.20	1.50	1.50	1.50
L-lysin	0.50	0.50	0.50	0.50
DL- Methionin	0.20	0.25	0.20	0.25
Nutrient				
Dry Matter (%)	98.84	99.13	98.80	99.15
Ash Content (%)	5.34	5.33	5.28	5.29
Crude Protein (%)	22.02	19.54	22.02	19.57
Ether Extract (%)	4.69	4.82	6.53	6.67
Crude Fiber (%)	4.23	4.31	4.58	4.69
Metabolizable Energy (Kcal/kg)	3094.35	3091.13	3095.07	3091.17
Calcium (%)	1.05	1.04	1.04	1.06
Total phosphorous (%)	0.55	0.59	0.57	0.56
Phosphor available (%)	0.40	0.42	0.41	0.40
Lysine (%)	1.30	1.27	1.33	1.28
Methionine (%)	0.57	0.57	0.56	0.56

CP = Crude Protein

Table 2. The concentration trypsin inhibitor in some ingredients

Ingredients	Trypsin Inhibitor Concentration (mg/g Ingredient)
Soybean flour	10.17
Soybean meal	5.65
Jack bean seed flour	9.34
Jack bean seed heated by autoclave	7.96

Metabolizable Energy

Metabolizable energy was measured using a modified Farrel (1978) method with the principle of total excreta collection. On days 22-23, Broiler chickens were moved to the metabolic cage and adapted by feeding the experimental ration. On day 24, Broiler chickens fasted but there was no restriction to access the drinking water. On days 25-28 Broiler chickens were fed for 1 hour, except the chickens assigned for endogenous losses assay, and their excreta were collected every 24 hours. Every 3 hours, the excreta were sprayed with sulfuric acid 0.01 N. The collected excreta were stored and frozen in the freezer for 24 hours. After that, the thawing process was carried out and the excreta were oven-dried at 60° C. The dried excreta were grounded and were being gross energy and crude protein content analyzed then the data obtained were calculated for the metabolic energy assay.

Experimental Design and Data Analysis

The experimental design used was a factorial completely randomized design (FCRD). There were 3 factors as treatments, including the type of protein source (soybean meal and autoclaved jack bean seed meal), the protein levels (22% and 19.5%), and the enzyme treatments (with and without the addition of the protease enzymes). This study consisted of 3 replications with each replication consisting of 10 male broiler chickens. Data were analyzed by Analysis of Variance (ANOVA) in accordance with Steel and Torie (1993).

RESULT AND DISCUSSION

Performance

The use of Jack bean seed meal in starter phase broiler chicken ration had no effect on feed intake, body weight, and FCR but significantly (P <0.01) decreased the value of body weight gain. The decrease in BWG of starter phase broiler chickens fed with Jack bean seed meal could be improved with the inclusion of protease enzymes up to the same level of performance in chickens fed with soybean meal-based basal ration. The low protein content with 19.5% in the ration significantly (P <0.01) decreased the BWG of broiler chickens in the starter phase, but it also could be increased with the addition of protease enzymes up to the same level of performance in chickens fed with ration containing adequate protein content as in 22%. The use of protease enzymes in the starter phase of the broiler chicken ration significantly (P <0.01) increased body weight and body weight gain. There was no interaction between feeding jack bean seed meal, level of protein content in the ration, and inclusion of protease enzyme on the performance of broiler chickens. The data of the performance of broilers in the starter phase was presented in Table 3.

The performance of broiler chickens in this study was still below the performance standards. According to Suasta (2019), the bodyweight of starter broilers that fed basal feed was maintained with a close house system of 870 g with BWG of 830 g and feed intake of 1,245 g so that the FCR value is 1.50. In this study, the weight of broiler chickens obtained was around 275 - 322 g with BWG of 228-254 g and feed intake of 441 - 542 g so that the FCR was 1.73 - 2.06. The low-performance result in this study might be due to the uncomfortable environment of the broiler chickens because they were caged in a semi-open house system and were assumed to suffer necrotic enteritis throughout their physical symptoms.

The feed intake of broiler chickens in this study was not significantly different within treatment factors due to the experimental rations had already sufficient energy content. One of the factors that influenced the feed intake was the compliance of the energy value, broiler chickens fed with less energy ration tended to consume more to fulfill their requirement. According to Razak (2016), feeding with the same energy and protein ratio would produce identical feed intake values. A feeding ration with less energy content would increase the amount of feed intake (Silondae, 2018).

The incorporation of Jack bean seed meal in the broiler chicken ration significantly (P <0.01) decreased the BWG of broiler chickens. The rations that contained 7% of Jack bean seed meal were digested slower in the digestive tract compared to soybean meal-based ration. Jack bean seed meal contained anti-nutritional trypsin inhibitors bound to protein and interfered with the protein absorption in the body. Trypsin inhibitor is a type of protein antinutrient that inhibits the action of the trypsin enzyme in the body and the protein absorption will be inhibited (Liu 1999). In line with Liu (1999), a trypsin inhibitor level of 7.6 mg/kg was detected and presented in Table 2. According to Lukiwati and Prawiradiputra (2014), the antinutrient content in the form of trypsin inhibitor in jack bean seed meal was 8.9 units per gram. This decrease in body weight gain and could be overcome by the addition of enzymes to the ration. The use of protease enzymes significantly (P <0.01) improved the body weight gain performance of starter-phase broilers. Broilers in the starter phase fed with a 7.5% jack bean seed meal-contained ration with the addition of protease enzymes could result in a similar BWG with soybean meal-based-basal ration. The use of enzymes could increase the digestibility of protein in the ration. Protease enzymes helped to break down the complex polypeptides into simpler peptides to be easily absorbed by the body (Sari, 2007).

Table 3. Performance of Broiler chicken starter phase

Material	Level of CP (%)	Enzyme		Average of Material	Average of Protein level	
		Without enzyme	Protease			
			Feed Intake (g)			
Basal	22	490.67 ± 8.40	522.06 ± 56.10		22 (%)	496.23±46.32
Basal	19.5	500.37 ± 15.08	527.95 ± 55.55	510.26±37.98		
Jack bean	22	486.33± 4.10	485.85 ± 12.26	477.12±52.91	19.5 (%)	491.15±52.91
Jack bean	19.5	456.61 ± 71.47	479.69 ± 53.19			
	Average	483.49 ± 35.73	503.89 ± 46.32			
			BW (g)			
Basal	22	302.20 ± 11.46	332.18 ± 12.41	302.19±23.66	22 (%)	308.07±19.26B
Basal	19.5	274.91 ± 16.65	299.47 ± 16.65			
Jack bean	22	284.01 ± 19.23	313.89 ± 18.81	290.34±20.61	19.5 (%)	284.04±15.71A
Jack bean	19.5	275.03 ± 15.45	288.44 ± 28.06			
	Average	284.04 ± 15.71A	308.07 ±22.08B			
			BWG (g)			
Basal	22	253.08 ± 10.12	285.50 ± 12.42	258.36±21.76B	22 (%)	260.40±12.53B
Basal	19.5	231.62 ± 8.58	263.25 ± 7.68			
Jack bean	22	236.32 ± 15.54	266.69 ± 6.54	240.18±23.23A	19.5 (%)	238.14±23.19A
Jack bean	19.5	216.45 ± 15.68	241.26 ± 27.20			
	Average	234.37 ± 17.44A	264.17 ±20.12B			
			FCR			
Basal	22	1.94 ± 0.09	1.83 ± 0.21	1.99 ± 0.20	22 %	1.91 ± 0.18
Basal	19.5	2.17 ± 0.17	2.01 ± 0.21			
Jack bean	22	2.06 ± 0.08	1.82 ± 0.01	2.00 ± 0.21	19.%	2.07 ± 0.20
Jack bean	19.5	2.11 ± 0.26	2.00 ± 0.19			
	Average	2.07 ± 0.18	1.91 ± 0.20			

CP= Crude Protein, BW= Body Weight, BWG= Body Weight Gain, FCR = *Feed Conversion Ratio*, different capital letters in the same row or column indicate significantly different (P<0.01).

The low quality of the Jack bean seed meal's protein could be improved by the inclusion of the protease enzyme. The increase in protein digestibility would also increase the BW. Protein is utilized by the body to compile body tissues and it would increase body growth (Wahyu, 2004). The increase in protein digestibility in the ration was conveyed by an increase of nitrogen-corrected true metabolizable energy as shown in Table 4 of 100.43 Kcal/kg although it was not statistically significant. Enzymes would improve digestion by catalyzing the breakdown of proteins into

DOI: 10.21776/ub.jiip.2021.031.03.03

simpler forms and easily absorbed by the body. This is in line with the study of Peric et al. (2008), the addition of enzymes in ration had a positive impact on growth and feed conversion ratio in broilers. The FCR in this study did not show any difference. Although statistically, it was not significantly different, a slight change in the FCR might have an impact on the profit of the business in large-scale industries. The FCR showed the efficiency of the feed, the higher the FCR value, the lower the efficiency.

Although it was not statistically different, the data showed a slight difference in the FCR value. The ration containing 7% jack bean seed meal showed a higher FCR value. This was due to the Jack bean seed meal containing trypsin inhibitors antinutrients that interfered with the protein absorption. The decrease in protein content in the ration tended to increase the FCR.

Protease enzyme inclusion could slightly improve the FCR because this enzyme worked in the process of breaking down protein into amino acids to be easily absorbed by the body.

Metabolizable Energy

The incorporation of 7% Jack bean seed meal in the broiler chicken ration significantly (P <0.01) increased all the metabolizable energy of the ration. The decrease in protein level in the ration of Broiler chicken in the starter phase significantly (P <0.01) decreased the value of true metabolizable energy and nitrogen corrected-true metabolizable energy. There was no interaction between the use of Jack bean seed meal, protein levels, and the inclusion of the protease enzyme in the ration on the result of metabolizable energy assay. The data of the metabolizable energy assay in broiler chickens aged 25-28 days were shown in Table 4.

The metabolizable energy of broiler chickens fed with a ration containing 7% jack bean seed meal was significantly (P <0.01) higher than those were fed with basal ration due to the crude fat content of the ration containing jack bean seed meal being higher. Apart from crude fat, a supplementary effect of several nutrients, especially protein, was assumed. The inclusion of the protease enzyme could improve the protein digestibility even though it did not affect significantly. The increase in digestibility would increase nitrogen retention. Protease is an enzyme that accelerates the breakdown reaction of peptide bonds in polypeptides and proteins by using a hydrolysis reaction to be simpler molecular molecules such as short-chain peptides and amino acids (Naiola and Widyastuti, 2014).

The increased protein digestibility and nitrogen retention not only would increase the AMEn and TMEn values but also would accelerate the process of gluconeogenesis, which is a reaction of converting non-carbohydrate compounds into energy needed by the body. The main substrates of gluconeogenesis are glucogenic amino acids, lactic acid, glycerol, and propionate acid (Sari, 2009). The use of protease significantly (P <0.01) increased the metabolizable energy of broiler chickens fed with corn, rice bran, and soybean meal-based ration. The inclusion of 15,000 units per kg of protease enzymes in the wheat and soybean meal-based ration of duck could increase the energy intake as in the value of AME and AMEn (Yuan, 2008).

The metabolizable energy of broiler chickens fed with a low protein level significantly (P <0.01) decreased the TME and TMEn compared to those fed with an adequate protein-contained ration by the standard requirements. Protein deficiency in an organism would interfere with the metabolism processes in the body. The data showed that the inclusion of the protease enzyme in the ration containing low protein levels was not significantly different from the ration formulated according to its standard requirement. The use of the protease enzyme was able to overcome the problem of low metabolizable energy in broiler chickens that fed with a low protein level of ration due to the increase of the metabolizable energy and approached to the metabolizable energy value in basal ration that formulated to their requirements. Protease enzymes indeed aim to improve protein digestibility.

Table 4. Metabolizable Energy (Kcal/kg) of broiler chicken 25-28 day old

Material	Level of CP (%)	Enzyme		Average of material	Average of CP Level	
		Without Enzyme	Protease			
AME (Kkal/kg)						
Basal	22	2757.35 ±226.95	2981.21 ± 35.91	2549.13±	22%	2761.15 ± 191.70
Basal	19.5	2567.77 ± 44.35	2775.35 ± 231.92	100.04A		
Jack bean	22	2884.59 ±347.51	2982.77 ± 72.02	2854.28	19.5%	2642.26 ± 199.01
Jack bean	19.5	2700.57 ±375.18	2849.18 ± 266.61	±117.021B		
	Average	2642.15 ±199.01	2761.15 ± 191.70			
TME (Kkal/kg)						
Basal	22	2896.22 ±215.19	2981.21 ± 35.91	2852.53±	22%	3117.36 ±
Basal	19.5	2757.35 ±277.33	2775.35 ± 231.91	105.64A		260.76A
Jack bean	22	3350.13 ±386.03	3403.54 ± 64.18	3297.35±	19.5%	3032.52 ±
Jack bean	19.5	3126.39 ±252.34	3309.34 ± 104.19	120.33B		260.76B
	Average	3032.52 ±260.76	3117.36 ±291.12			
AMEn (Kcal/kg)						
Basal	22	2562.15 ±227.16	2652.77 ± 85.91	2543.00±	22%	2754.76 ± 191.32
Basal	19.5	2409.32 ± 92.41	2547.75 ± 83.42	100.52A		
Jack bean	22	3350.13 ±386.03	3403.54 ± 64.18	2847.32±	19.5%	2635.55 ± 198.47
Jack bean	19.5	3126.39 ±252.34	3309.34 ± 104.19	92.41B		
	Average	2635.55 ±198.47	2754.76 ±191.32			
TMEn (Kcal/kg)						
Basal	22	2890.60±278.05	2975.50±58.15	2870.14±	22%	3151.21±
Basal	19.5	2750.63±35.78	2750.63 ± 43.66	206.30A		250.13A
Jack bean	22	3342.32±391.99	3396.40 ± 64.23	3151.2 ±	19.5%	3025.83 ±
Jack bean	19.5	3119.77±254.19	3302.74 ± 105.45	255.23B		260.14B
	Average	3025.83±260.14	3126.26 ±267.31			

CP = Crude Protein, AME = Apperent metabolizable Energy, TME= True Metabolizable Energy, AMEn = Apperent metabolizable Energy correcten Nitrogen, TMEn= True Metabolizable Energy corrected nitrogen

An increase in protein digestibility would have an impact on the metabolizable energy because the gluconeogenesis process occurred properly.

The process of gluconeogenesis is the process of breaking down the non-carbohydrate compounds into glucose. One of these non-carbohydrate compounds and part of the crude protein is glucogenic amino acids (Sari, 2009).

CONCLUSIONS

The decrease in protein level in the ration reduced the performance and metabolizable energy of broiler chickens in the starter phase. That negative effect could be improved with the inclusion of protease enzymes and the performance would be enhanced up to the same rate as the performance fed with a soybean meal-based basal ration. On the other hand, the inclusion of protease enzymes in the ration could improve the performance of broilers in the starter phase.

The incorporation of 7% jack bean seed meal in the ration could reduce the BWG of broiler chickens and it could be improved by the inclusion of the protease enzyme with approximate results to the birds fed with basal ration. The incorporation of a 7% jack bean seed meal in the ration of broiler chicken in the starter phase could increase the value of the metabolizable energy.

DOI: 10.21776/ub.jiip.2021.031.03.03

REFERENCES

Canadian Bio System. (2015). *Concentrase-P*.

Farrell, D. J. (1978). Rapid determination of metabolisable energy of foods using cockerels. *British Poultry Science*, *19*(3), 303–308. https://doi.org/10.1080/00071667808416480

Hilyati, H., & Etika, S. B. (2017). Kerentanan tripsin-inhibitor biji turi terhadap panas. *AgriTECH*, *11*(1), 2–10. https://doi.org/10.22146/AGRITECH.19214

Kasno, A. (2016). *Prospek Aneka Kacang Potensial: Koro Pedang sebagai Pengganti Kedelai. Balai Penelitian Kacang dan Ubi*.

KEMENDAG (Kementerian Perdagangan). (2017). *Perkembangan Impor Menurut HS 6 Digit Periode : 2012-2017*. Kementerian Perdagangan RI.

Leeson, S., & Summers J, D. (2005). *Commercial Poultry Nutrition* (3rd ed.). Nottingham University Press.

Liu, K. (1999). *Soybean: Chemistry, technology, and utilization*. Aspen Publ., Inc.

Lukiwati, D, R., & Prawiradiputra, B, R. (2014). *Peluang koro pedang sebagai pangan, pakan, dan tanaman obat*. Universitas Diponegoro.

Naiola, & Widhyastuti. (2002). Isolasi, seleksi dan optimasi produksi protease dari beberapa isolat bakteri. *Berita Biologi*, *6*(3), 467–473.

Patrick, H., & Schaible, P. J. (1980). *Poultry Feed and Nutrition*. Aci Publ., Co., Inc.

Primawestri, M. A., & Rustanti, N. (2014). Pengaruh pemberian susu koro pedang (Canavalia ensiformis) terhadap kadar kolesterol total dan trigliserida serum tikus sprague dawley hiperkolesterolemia. *Journal of Nutrition College*, *3*(4), 447–455. https://doi.org/10.14710/jnc.v3i4.6826

Razak, A. D., Kiramang, K., & Nurhidayat, M. N. (2016). Pertambahan bobot badan,konsumsi ransum dan konversi ransum ayam ras pedaging yang diberikan tepung daun sirih (Piper Betle Linn) sebagai imbuhan pakan. *Jurnal Ilmu Dan Industri Peternakan*, *3*(1), 135–147.

Sari, I. M. (2009). *Reaksi Reaksi Biokimia Sebagai Sumber Glukosa Darah*. Universitas Sumatera Utara.

Silondae, H., & Polakitan, D. (2018). Pengaruh imbangan energi dan protein serta kepadatan kandang terhadap penampilan ayam pedaging. *Jurnal Peternakan Indonesia (Indonesian Journal of Animal Science)*, *20*(3), 175–180. https://doi.org/10.25077/jpi.20.3.175-180.2018

Soetrisno, U. S. S., & Suryana, P. (1991). Pengembangan prosedur analisis zat antitripsin (trypsin inhibitor) pada sumber protein nabati. *Penelitian Gizi Dan Makanan (The Journal of Nutrition and Food Research)*, *14*, 153–158. https://doi.org/10.22435/PGM.V0I0.2216

Sridhar, K. R., & Seena, S. (2006). Nutritional and antinutritional significance of four unconventional legumes of the genus Canavalia – A comparative study. *Food Chemistry*, *99*(2), 267–288. https://doi.org/10.1016/j.foodchem.2005.07.049

Steel, R. G. D., & Torrie, J. H. (1993). Prinsip dan Prosedur Statistika: Suatu Pendekatan Biometrik. In B. Sumantri (Ed.), *PT Gramedia Pustaka Utama* (1st ed., Issue 5004). Gramedia Pustaka Utama.

Suhartono, M. . (2000). Pemahaman Karakteristik Biokimia Enzim Protease dalam Menunjang Industri Berbasis Bioteknologi. In *Buku Orasi Ilmiah Guru Besar Ilmu-Dasar Biokimia Dasar*. Institut Pertanian Bogor.

Wahju, J. (2004). Ilmu Nutrisi Ternak Unggas. In *Gadjah Mada Universitas Press, Yogyakarta* (5th ed.). Gadjah Mada University Press.

Yuan, J., Yao, J., Yang, F., Yang, X., Wan, X., Han, J., Wang, Y., Chen, X., Liu, Y., Zhou, Z., Zhou, N., & Feng, X. (2008). Effects of supplementing different levels of a commercial enzyme complex on performance, nutrient availability, enzyme activity and gut morphology of broilers. *Asian-Australasian Journal of Animal Sciences*, *21*(5), 692–700. https://doi.org/10.5713/ajas.2008.70375