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Original Article

The evaluation of the use of jack bean (*Canavalia ensiformis*) and protease enzyme on the broiler diet with the different level of protein

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Abstrak

Tujuan: Tujuan dilakukan penelitian ini untuk mengevaluasi penggunaan kacang koro pedang (*Canavalia ensiformis*) dan enzim protease dalam pakan ayam broiler taraf protein berbeda terhadap karkas, dan efisiensi ransum yang dinilai berdasarkan protein efisiensi ratio, retensi nitrogen, persentase organ imunitas dan analisis ekonomi pakan berdasarkan *Income Overfeed Convertion*.
Metode: Rancangan Penelitian yang digunakan dalam penelitian ini adalah Rancangan Acak Lengkap Faktorial (RAL) dengan 3 faktor perlakuan diantaranya level protein (22% dan 19,5%), bahan sumber protein (pakan basal dan koro pedang), dan penggunaan enzim (tanpa enzim dan suplementasi protease) dan 3 kali ulangan (terdiri dari 2 sampel ayam broiler setiap ulangan). Ternak yang digunakan adalah ayam broiler jantan sebanyak 48 ekor sampel yang diambil dari 240 ekor populasi yang sebelumnya telah dipelihara selama 28 hari.
Hasil: Hasil penelitian menunjukkan bahwa penggunaan kacang koro pedang secara nyata (P<0,05) menurunkan bobot hidup, bobot potong dan bobot karkas dan secara sangat nyata (P<0,01) meningkatkan IOFC. Penggunaan enzim protease secara nyata (P<0,05) meningkatkan retensi nitrogen, *protein efisiensi ratio* dan karkas. Penurunan level protein pakan secara sangat nyata (P<0,01) menurunkan konsumsi protein, RN, PER, Bobot hidup, bobot potong, dan karkas. Penggunaan koro pedang tidak mempengaruhi ukuran organ imunitas broiler.
Kesimpulan: Penggunaan enzim protease dalam pakan level protein rendah (19,5) dapat menghasilkan bobot hidup , karkas, RN dan PER sama dengan pakan basal tanpa mempengaruhi ukuran organ imunitas ayam broiler.

Kata Kunci: Jack bean; Protease; Protein level

Abstract

Objective: This study evaluated the use of jack beans (*Canavalia ensiformis*) and protease enzymes in broiler chicken feed. The protein levels on carcasses and the efficiency of the diet were assessed based on protein efficiency ratio, nitrogen retention, percentage of immunity organs, and feed economic analysis based on Income Overfeed Conversion.

Methods: The factorial complete randomized design (CRD) was employed in this study, piloting three treatment factors: protein level (22% and 19.5%), protein source material (basal diet and jack bean), and the use of enzymes (without enzymes and protease supplementation). Moreover, three replications were conducted, consisting of two samples of broiler chickens per replication. Forty-eight male broiler chickens from 240 populations that had previously been reared for 28 days were taken as the sample of the study.
Result: The results showed that the use of jack beans significantly decreased the dress body weight (P<0.05), undress body weight, and carcass weight of broiler chickens. In addition, the weight significantly increased IOFC by (P<0.01). The use of protease enzymes significantly (P<0.05) increased Retention Nitrogen, Protein Eficiency Ratio, and carcass. The feed protein level decreased substantially by (P<0.01). It reduced protein consumption, RN, PER, dress, undress, and carcass body weight. The use of jack bean did not affect the broiler's immune organ size.
Conclusion: The use of protease enzymes in diets with low protein levels can produce dress body weight, carcass body weight, RN, and PER, similar to basal diets, without affecting the size of the immune organs of broiler chickens.

Keywords: Jack bean; Protease; Protein level

INTRODUCTION

Poultry feed, especially broiler chickens, is still very dependent on imports for protein fulfillment. Feed for poultry, especially broilers in Indonesia, is based on soybean meal as a source of protein. The availability of soybean meal in Indonesia is still utterly dependent on imports. The soybean meal price is relatively high, so it will increase the diet cost if local protein sources are not sought as alternatives. A local feed ingredient that can be used as an alternative protein source for soybean meal is the jack beans (*Canavalia ensiformis*). The availability of jack beans in Indonesia is quite a lot, namely the land for planting jack beans of 1590 ha with production reaching 5 tons/ha/year. Jack bean has a good nutrient content, namely crude protein content reaching 30% to 36.40%, ash (3.5%-5.32%), lipid (5.85%-9.23%), fiber (3.25%-6.35%) [1]. In addition, the jack bean has an antioxidant activity of 0.984 mcg/ml, vitamin C 8087 mg/100g [2].

Jack beans are not recommended to be used in high quantities and raw without processing them in animal feed because they contain trypsin inhibitors. The trypsin inhibitor level of jack bean was 9.39 mg/g of material, whereas if it was heated using an autoclave, the trypsin inhibitor level decreased to 7.96%. The use of jack beans on 7% in broilers diet aged 28 days can increase pure metabolic energy and nitrogen-corrected pure metabolic energy. The jack beans do not affect broiler performance. Therefore, they can be used as feed ingredients for local protein sources [3,4] stated that the jack beans with oaked with water at room temperature for 24 hours then boiled for 20 minute could be used as a substitute for 50% soybean meal protein without affecting the performance, carcass, and internal organs of 35-day-old broiler chickens. [5] stated that using jack beans did not affect the size of broiler chickens' digestive and accessory organs.

Broiler chicken feed is often added with Antibiotic Growth Promoter (AGP) to improve feed and rearing efficiency. AGP can act as an agent to eradicate pathogenic microbes in the digestive tract and as a growth promoter. The use of AGP has many benefits on feed efficiency and livestock production but also harms livestock and saves residues in livestock products, such as its meat and offal. [6]. In addition, AGP may cause resistance to pathogenic microbes in livestock if given the wrong dose for an extended period [7,8]. Therefore, the Indonesian government, through Law number 18 of 2008 junco no 41/2014 article 22 paragraph 4C stated that everyone is prohibited from using materials mixed with certain hormones or antibiotics as supplements. Implementation of the law began on January 1, 2018.

An alternative to using AGP to increase diets' efficiency is using enzymes. The enzyme is a protein compound that is a biocatalyst.

Enzymes may work if they find a suitable substrate [9]. Enzymes are given to feed to improve livestock performance [10]. One enzyme that plays a role in feed efficiency is the protease enzyme. The use protease in the diet were no significant of broiler growth performance compare with AGP avilamycin [11]. Protease enzymes are divided into endogenous and exogenous enzymes. Endogenous enzymes are enzymes produced by the body, while exogenous enzymes are supplemented from outside the body or through the feed. Exogenous enzymes have been commercially used in monogastric animal feed to improve performance [12]. Protease enzymes are widely used and produced commercially, especially when the price of soybean meal is high [13]. Based on [3] the use protease can decrease FCR of broiler from 2.06 to 1.91. The use of proteases can reduce feed costs. The evaluations have also been carried out to increase protein and amino acid digestibility [14-16]. According to [3] the use of protease enzymes was able to make feed with low protein, resulting in the same performance of broilers aged 28 days with the given basal feed broiler. They meet the standard protein requirements. The protease enzyme increased feed consumption and nitrogen retention of quail fed containing Indigofera sp [17].

The use of jack beans (*Canavalia ensiformis*) as a substitute for soybean meal with the addition of protease enzymes is expected to increase feed efficiency and broiler livestock production. The efficiency of feed use, especially the use of feed protein, can be measured based on nitrogen retention levels and protein efficiency ratios. Furthermore, production efficiency can be seen from the percentage of the leading livestock product, namely the weight of the carcass produced by the livestock compared to its live weight. Apart from feed and production efficiency, livestock health needs to be evaluated by adding jack beans and protease enzymes. One of the health livestock evaluations can be done by measuring the percentage of their immune organs. Based on this background, this study was conducted to evaluate the use of jack beans and protease enzymes in broiler chicken feed with different protein levels on feed efficiency, production, and immune organs of broiler chickens.

MATERIALS AND METHODS

Instruments and materials

Alumunium Knives, surgical scissors, tweezers, plastic trays, weighing scales, and stationery were the instruments measuring carcasses and organs of immunity. IOFC using the Microsoft Excel application and data analysis using the SPSS application version 16 were situated to analyze the feed economy. Finally, feed ingredients for broiler diet in the form of corn, soybean meal, Corn Gluten Meal (CGM), jack beans, CaCO3, DCP, premix, and protease enzymes were the used materials in this study. The vitamin used in this study was Vitastress.

Bird and housing

The Bird used was Lohmaan Strain Broiler Chicken. Forty-eight from 240 broiler chickens were taken as research samples reared from DOC (Day old Chick) until 28 days of rearing. A flock of broiler chickens was reared in a cage of 1x1 m with a capacity of 10 chickens per cage. The cage was equipped with litter or padding made of rice husks, a place to eat, a place to drink, a brooder from zenk, and a 100-watt light bulb as a heater.

Jack beans (*Canavalia ensifromis*)

The jack beans used in this study were the jack beans that had been ground into powder. The flour was treated as an autoclave for 25 minutes at a temperature of 26 °C and a pressure of 1 atm. It was then analyzed proximately and formulated with a level of 7% in feed and mixed with other feed ingredients to be used as diet treatment. The use of jack beans in the treatment ratio was 7%. The data on the nutrient content of jack beans are presented in Table 1.

Feed

The research feed was formulated according to the recommendations of [17] and the reduced protein content was compared to the needs of broiler chickens. The feed is iso protein fed. The nutrient content of the formulation results and the results of the proximate analysis were presented in Tables 2 and 3.

Table 1. Results of chemical analysis of jack beans (*Canavalia ensiformis*)

Chemical content	Content
Dry matter (%)	98.8
Moisture (%)	1.2
Ash content (%)	3.69
Crude Protein (%)	27.46
Crude Fiber (%)	11.51
Ether Extract (%)	3.51
Calcium (%)	0.13
Phospor (%)	0.02
Trypsin Inhibitor (mg/g)	7.96

Result of Laboratory analyist of feed science and technology IPB University

Enzim protease

The protease enzyme used in this study was a commercial protease enzyme produced by PT Canadian Biosystem. The protease enzyme was obtained from the extract of Lactobacillus ensiformis. The activity of the protease enzyme was 12500 HUT/g. The ingredients for the protease enzyme were wheat flour, lime, and silicon dioxide as coupling enzymes. They were used to avoid vulnerability while making feed into pellets and crumbles. The dose of protease enzyme was 0.5 g per kg of feed.

Reseach procedure

In this study, it was started from rearing livestock from Day Old Chick to 28 days old chicks or the grower period. Treatment is given at the age of 8 days or the starter period. The number of livestock used during rearing was 280 which were distributed to each replication for each treatment factor. In this research, protein efficiency ratio, nitrogen retention, IOFC carcass percentage and Immunity organ were measured. The number of livestock samples used for measuring nitrogen retention and carcasses was 2 in each replication.

Measurement of retention nitrogen is calculated by the formula :

$$\text{RN} = \frac{(\text{FC X NC}) - (\text{E X NE})}{(\text{FC X NC})} X\ 100\%$$

Table 2. Nutrient content of reserch diet based on formulation

Nutrient Content	Basal CP 22%	Basal CP 19.5%	Jack Beans CP 22%	Jack Beans CP 19.5%
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 3. Results of research diet chemical analysis

Diet	DM	Moisture	Ash	CP	EE	CF	Ca	P
Basal CP 22%	89.15	10.85	5.35	22.49	7.22	5.42	0.99	0.52
Basal CP 19.5%	89.20	10.80	5.85	20.03	7.49	5.91	0.86	0.57
Jack bean CP 22%	89.77	10.23	5.85	22.40	7.03	6.29	1.18	0.43
Jack bean CP 19.5%	89.44	10.56	5.79	19.92	7.45	6.18	1.01	0.37

DM= Dry matter, CP = Crude protein, EE = Ether extract, CF = Crude fiber, Ca=Calsium, P = Phospore

RN = Retention of Nitrogen, FC = Feed Consumption, FN = Feed Nitrogen, E = Excreta Weight , NE = Nitrogen Excreta

Measurement of Protein Efisiensy Ratio is calculated by the formula:

$$\mathrm{PER} = \frac{\mathrm{BWG}}{\mathrm{FC\ x\ CP}} X\ 100\%$$

PER = Protein Eficiency Ratio, BWG = Body Weight Gain, Fc = Feed Consuptiom, CP Crude Protein

Measurement of Income Overfeed Cost is calculated by the formula:

$$\mathrm{IOFC} = \mathrm{Income} - \mathrm{total\ feed\ Cost}$$

Carcass percentage is calculated by the formula:

$$\mathrm{Carcass} = \frac{\mathrm{Carcass\ Weight}}{\mathrm{Live\ Body\ Weight}} X\ 100\%$$

Measurement of Percentage of Immunity Organ

$$\mathrm{Immunity\ organ} = \frac{\mathrm{Weight\ of\ Organ}}{\mathrm{Live\ Body\ Weight}} \times\ 100\%$$

Design and data analysis

A factorial complete randomized design (RALF) was deployed, plotting three factors and three replications (two samples of broilers per replication). The first factor was protein level, which included providing protein (22%) according to the legal needs of livestock and low protein (19.5%). The second factor was the provision of enzymes (feed without adding enzymes and feed with the addition of proteases). The third factor was the protein source material that made up the diet (basal feed of soybean meal and 7% addition of jack beans). The Analysis of Variance (ANOVA) using the SPSS application version 16 was deployed to analyze the data.

RESULTS

The effect of treatment on protein utilyzation

Data on protein consumption, protein efficiency ratio, and nitrogen retention of broiler chickens fed with different protein levels containing jack beans and protease enzymes were presented in Table 4.

The decrease in protein level in the feed was very significant (P<0.01). It decreased RN but did not significantly affect protein

Table 4. Protein consumption, protein ratio efficiency, and nitrogen retention of broiler chickens fed with different protein levels containing jack beans and protease enzymes

Feed ingredients	Level	Enzyme: Non Enzim	Enzyme: Protease	Feed ingredients	Average level	
Protein consumption (g)						
Basal	22	107.95 ± 12.34	114.85 ± 12.34	106.04 ± 9.77	22%	109.17 ± 6.36
Basal	19.5	97.57 ± 2.95	102.95 ± 10.83			
Jack bean	22	106.99 ± 0.90	106.89 ± 0.50	99.11 ± 11.16	19.50%	95.78 ± 10.32
Jack bean	19.5	89.04 ± 13.94	93.54 ± 10.37			
Enzyme average		104.56 ± 11.53	100.39 ± 10.12			
Nitrogen retention (%)						
Basal	22	87.04 ± 5.40	90.59 ± 3.11	85.88 ± 3.84	22%	87.32 ± 2.52A
Basal	19.5	81.62 ± 9.01	84.27 ± 9.51			
Jack bean	22	84.48 ± 1.16	87.17 ± 0.27	83.43 ± 4.18	19.50%	81.99 ± 3.24B
Jack bean	19.5	77.57 ± 1.21	84.48 ± 1.93			
Enzyme average		82.68 ± 4.01a	86.63 ± 2.95b			
PER						
Basal	22	2.51 ± 0.28	2.35 ± 0.10	2.46 ± 0.22	22%	2.39 ± 0.18
Basal	19.5	2.38 ± 0.20	2.58 ± 0.29			
Jack bean	22	2.21 ± 0.08	2.50 ±0.01	2.44 ± 0.25	19.50%	2.50 ± 0.27
Jack bean	19.5	2.47 ± 0.40	2.59 ± 0.25			
Enzyme average		2.35 ± 0.21a	2.54 ± 0.21b			

The notation at the end of the numbers indicates significantly different treatment (P<0.05) consumption and PER value. The use of jack beans in the diets had no significant effect on protein consumption, RN, and PER. The use of protease enzymes significantly (P<0.05) increased RN and PER but did not substantially affect protein consumption. There was no interaction between protein levels, jack beans feed ingredients, and protease enzymes on protein consumption, RN, and PER.

The effect of treatment on broiler carcass production

Dress weight, undress weight, and carcass production data of broiler chickens fed with different protein levels containing jack beans and protease enzymes were presented in Table 5.

Feed with a low protein level (19.5%) significantly (P<0.01) reduced dress weight, undress weight, carcass weight, and carcass percentage to dress weight. The use of jack beans significantly (P<0.05) reduced dress weight, undress weight, carcass weight, and carcass percentage to dress weight. Adding protease enzymes in the feed significantly increased carcass weight and percentage to dress weight. There was no interaction between the decrease in protein level, the use of feed ingredients containing jack beans, and protease enzymes. Dress weight in this study ranged from 255.83-353.83 g.

The effect Treatment on Income Overfeed Conversion (IOFC).

The IOFC data of broiler chickens fed a diet containing jack beans (*Canavalia ensiformis*) and protease enzymes in the diet of different protein levels were presented in Table 6.

The use of jack beans (*Canavalia ensifromis*) significantly increased the IOFC

Table 5. Dress weight, undress weight, and carcass production of broiler chickens fed with different protein levels containing koro beans and protease enzymes

Feed ingredients	Level	Enzyme		Feed ingredients	Average level	
		Non-enzyme	Protease			
Dress body weight						
Basal	22	298.83 ± 12.09	353.83 ± 14.89	298.23 ± 28.13b	22%	304.88 ± 23.63B
Basal	19.5	281.67 ± 20.14	286.67 ± 17.50			
Jack bean	22	296.67 ± 19.98	288.67 ± 17.50	279.88 ± 23.11a	19.50%	275.63 ± 23.14A
Jack bean	19.5	258.83 ± 11.62	275.33 ± 29.39			
Enzyme average		283.88 ± 21.71	296.63 ± 31.63			
Undress body weight						
Basal	22	286.33 ± 11.09	324.50 ± 11.09	286.57 ± 28.04b	22%	293.13 ± 24.26B
Basal	19.5	269.67 ± 19.47	275.67 ± 28.85			
Jack bean	22	276.67 ± 18.45	285.00 ± 21.50	267.88 ± 23.99a	19.50%	263.79 ± 23.41A
Jack bean	19.5	246.83 ± 12.57	263.00 ± 30.79			
Enzyme average		271.96 ±21.92	284.96 ± 32.14			
Carcase weight						
Basal	22	185.55 ± 6.90	219.80 ± 7.61	186.74 ± 23.80B	22%	192.06 ± 18.51B
Basal	19.5	168.33 ± 11.92	178.44 ± 25.01			
Jack bean	22	181.31 ± 11.02	181.59 ± 10.11	170.55 ± 16.96A	19.50%	166.52 ± 17.96A
Jack bean	19.5	151.01 ± 5.59	168.26 ± 19.79			
Enzyme average		171.55 ± 16.10a	187.02 ± 25.12b			
Carcase percentage						
Basal	22	62.23 ± 2.50	65.47 ± 0.93	62.49 ± 2.63B	22%	62.94 ± 2.05B
Basal	19.5	59.77 ± 0.55	62.08 ± 2.44			
Jack bean	22	61.13 ± 0.71	62.92 ± 0.49	60.87 ± 1.81A	19.50%	60.32 ± 1.87A
Jack bean	19.5	58.37 ± 0.86	61.06 ± 0.86			
Enzyme average		60.38 ± 1.93A	62.88 ± 2.08B			

The notation at the end of the numbers indicates significantly different treatment (P<0.05), capital letters indicate highly significant differences (P<0.01)

Table 6. IOFC of broiler chickens fed a diet containing jack beans (*Canavalia ensiformis*) and protease enzymes in different protein levels

IOFC						
Feed ingredients	Level	Enzyme		Feed ingredients	Average level	
		Non-enzyme	Protease			
Basal	22	2139.27 ± 293.23	2218.94 ± 340.67	2071.18 ± 565.18A	22%	2353.35 ± 424.49
Basal	19.5	1576.41 ± 386.43	1813.70 ± 344.17			
Jack bean	22	2447.60 ± 486.45	2607.60 ± 227.66	2473.75 ± 358.35B	19.50%	2057.48 ± 614.00
Jack bean	19.5	2526.79 ± 270.27	2313.01 ± 323.35			
Enzyme average		2172.52 ± 501.17	2238.31 ± 592.80			

The notation at the end of the numbers indicates significantly different treatment (P<0.05), capital letters indicate highly significant differences (P<0.01)

value of broiler chickens. The use of enzyme and protein levels did not substantially affect the IOFC value of broiler chickens. There was no interaction between the decrease in protein level, the use of feed ingredients containing jack beans, and protease enzymes.

The effect of treatment on the immune organs of broiler chickens

Data on the immunity organs of the bursa Fabricius, thymus, and spleen of broiler chickens fed with different protein levels containing jack beans and protease enzymes were presented in table 7.

The decrease in protein level, the use of jack beans in feed, and the use of protease enzymes did not significantly affect the immune organs of broiler chickens. In addition, this treatment indicated that it did not interfere with the livestock's immunity regarding the size of their internal organs.

DISCUSSION

The use of a diet containing jack beans on 7% reduced undress weight, dress weight, and carcass of broiler chickens because jack beans contain antinutrients in the form of trypsin

Table 7. Immunity organs of bursa fabricius, thymus, and spleen of broiler chickens fed with different protein levels containing jack beans and protease enzymes

Bursa Fabricius (%)						
Feed ingredients	Level	Enzymes		Feed ingredients	Average level	
		Non-enzymes	Protease			
Basal	22	0.20 ± 0.04	0.17 ± 0.05	0.17 ± 0.04	22%	0.19 ± 0.05
Basal	19.5	0.16 ± 0.04	0.13 ± 0.02			
Jack bean	22	0.25 ± 0.04	0.18 ± 0.03	0.22 ± 0.22	19.50%	0.18 ± 0.06
Jack bean	19.5	0.22 ± 0.01	0.21 ± 0.09			
Enzyme average		0.21 ± 0.06	0.18 ± 0.06			
Thymus (%)						
Basal	22	0.17 ± 0.05	0.16 ± 0.01	0.16 ± 0.02	22%	0.17 ± 0.05
Basal	19.5	0.15 ± 0.01	0.16 ± 0.01			
Jack bean	22	0.21 ± 0.09	0.14 ± 0.01	0.18 ± 0.05	19.50%	0.17 ± 0.03
Jack bean	19.5	0.21 ± 0.05	0.19 ± 0.01			
Enzyme average		0.17 ± 0.06	0.16 ± 0.05			
Spleen (%)						
Basal	22	0.13 ± 0.02	0.14 ± 0.02	0.13 ± 0.03	22%	0.12 ± 0.03
Basal	19.5	0.15 ± 0.03	0.10 ± 0.02			
Jack bean	22	0.11 ± 0.03	0.10 ± 0.03	0.12 ± 0.02	19.50%	0.13 ± 0.03
Jack bean	19.5	0.12 ± 0.02	0.13 ± 0.02			
Enzyme average		0.12 ± 0.03	0.13 ± 0.03			

The notation at the end of the numbers indicates significantly different treatment (P<0.05), capital letters indicate highly significant differences (P<0.01) inhibitor and Canavalin. [20] stated that jack beans have antinutrients, such as concanavalin A, canavanine, canaline, canatoxin, urease, saponins, and other toxic compounds. According to [19] the active compounds in the jack bean were choline, hydrozianine acid, troginelin and antichimotrypsin, phenols, isoflavones, niacin, and saponins. Trypsin inhibitors or antitrypsin inhibitors were secondary metabolites or natural compounds in grain and legume plants that function as self-defense from insects [20]. Trypsin inhibitor was an antinutrient that inhibited the work of proteins in the body [21]. According to [22] the use of raw soybean flour containing trypsin inhibitor of 6-21 mg/g of the material varied significantly ($P<0.01$) can reduce the body weight and feed conversion ratio of broiler chickens and significantly ($P<0.05$) reduce feed consumption. [23] also stated that high levels of antitrypsin (27.62 mg/g) in the feed may cause a decrease in feed digestibility.

The dress weight was smaller than the standard weight of broiler chickens, according to [24], which stated that the average body weight of broilers aged 28 days is 870 g. This difference in body weight was thought to be due to a digestive tract disease disorder, necrotic enteritis, at the beginning of the rearing. At the beginning of rearing, the chickens experienced diarrhea, calcareous defecation, bloody stools, and hair loss. The disease occurred allegedly due to maintenance using a semi-open house system with high humidity so viruses and bacteria could easily grow. Necrotic enteritis is caused by the bacterium C. perfringens. Necrotic enteritis attacks birds aged two to six weeks [19].

Although jack beans might reduce dress weight, undress weight, and carcass of broiler chickens, the economic calculations based on the IOFC and the use of jack beans in feed might increase the IOFC value. The increase in the IOFC value indicated that the income from the feed containing jack beans was higher than that from the basal diet. The unit price of jack beans protein was lower than soybean meal. According to [3] the price per unit of protein from jack beans was Rp. 127,460,00, while the soybean meal was Rp. 205, 510.00. IOFC was strongly influenced by diet consumption, final weight, diet price, and chicken price [23].

The use of jack beans had no significant effect on broiler immunity's PER, RN, and organ size. Based on these results, it can be said that the use of jack beans in the diet did not interfere with the protein digestibility and health of broiler chickens. Therefore, Jack beans had good protein value used as poultry feed ingredients. In this study, the crude protein content of jack beans reached 27.64% (Table 1). [4], stated that the use of jack beans replaced 50% of protein from soybean meal in the broiler diet . Therefore, it did not have a negative impact on the performance, PER, and internal organs of broiler chickens.

The use of protease enzymes in the broiler diet increased carcass' RN and PER because protease enzymes were protein compounds that were able to degrade proteins that all have complex structures into simpler ones so that they are easily digested and utilized by the body. Supplementation of exogenous proteases helped the degradation of bonds between proteins so that they could be digested by livestock. Besides that, proteases can also reduce the amount of protein stored in the body and make starch-rich bonds available to livestock [24]. protease can increase the digestibility of feed, especially protein so that poultry needs will be more easily met and poultry will feel full quickly so that feed consumption will decrease Proteolytic enzymes could break down disulfide bonds 56 and hydrolyze soluble casein, insoluble keratin, and other proteins crosslinked by disulfide 57 bonds [24]. The enzyme used in this study is an enzyme extracted from Bacillus ensiformis that degraded keratin and other types of proteins [25].

The use of protease enzymes in feeds with low protein levels resulted in RN, PER, carcass, and IOFC, which were not significantly different from standard protein levels. Based on these results, proteases could increase feed efficiency, so broiler chickens did not need to be fed too high protein feed to achieve good performance. According to [3] that the performance of broiler chickens fed with a low protein diet (19.5%) containing protease enzymes might be the same as high protein (22%). This statement was also in line with the opinion of [26] that the use of protease enzymes in low protein feed (19%) in broilers aged 29-48 days may produce the same performance as standard feed. [26] stated that adding proteases to low-protein diets can increase the availability or digestibility of nutrients. The performance of broiler chickens was strongly influenced by protein digestibility which was reflected in the RN and PER values. The more protein digested and absorbed, the better the chickens' growth. The protein functioned in the body was to build cells and body tissues [9] Using low protein feed that produced the same performance as high protein feed might also saved the cost of making feed, as reflected in the IOFC value (see table 6).

The use of low protein level feed (9.5%) reduced dress weight, undress weight, and carcass weight because protein deficiency caused disturbances in the metabolic system, immunity, and the growth of living things. According to [26] protein deficiency affected the depression of the immune system, which was related to the atrophy of the primary lymphoid organs that played a significant role in the immune system, namely the spinal cord and thymus. One of the fastest effects of atrophy in the thymus was leukopenia (decreased leukocyte count). According to [27] protein deficiency might adversely affected broiler chickens' performance and carcass and increase stress on broiler chickens. Protein is one of raw material for the formation of immune sytem. The deficeincy of protein can reduce the body's health.

In this study, the immune organs of the thymus, bursa Fabricius, and spleen were not significantly affected by the addition of jack beans, protein levels, and protease enzymes. The results indicated that treatment did not affect the livestock's immune system because the immune organs function properly and normally. Bursa Fabricius and thymus were primary lymphoid organs in poultry that regulated the production and development of lymphoid cells . Primary lymphoid organs acted as sites for the growth and development of immune cells [28] The thymus and bursa Fabricius organs were related to the production and function of T cells and B cells, which were cells that played a role in the livestock immune system. Bursa Fabricius is a primary lymphoid organ in charge of regulating the production and differentiation of B lymphocytes. The role of B lymphocytes as reactants and recipients of reactions to foreign objects that entered the body, such as free radicals or chickens experiencing heat stress. The spleen was one of the immunity organs that regenerate red blood cells (erythrocytes), form lymphocytes, and produce antibodies for the body [29]. The spleen had many roles in the process of hemoglobin formation, iron metabolism, infection prevention agents, red blood cell filtration, and platelet storage [30].

CONCLUSION

There was no interaction between protein levels, protein source feed ingredients and the use of protease enzymes but each factor showed a significant difference. Using jack beans (*Canavalia ensiformis*) in the broiler diet reduced broiler chickens' dress and carcass weight but increased IOFC. The use of protease enzymes in feed with different protein levels could increase feed protein efficiency, nitrogen retention, and broiler carcass weight. The use of protease enzymes in low protein feed was able to produce feed efficiency and production similar to the use of basal broiler feed. In addition, the use of jack beans and protease enzymes in feed did not interfere with the immune system of broiler chickens in terms of the size of their immune organs.

CONFLICT OF INTEREST

We (authors) state that the content addressed in this work has no conflict of interest with any financial entity. We further declare that we have no personal circumstances or interests that could be construed as improperly influencing the presentation or interpretation of stated research findings. Because this is a self-funded study, we declare that the funders had no involvement in the study design, data collection, analysis, interpretation, manuscript preparation, or the decision to publish the results.

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