The evaluation of protease enzyme effectiveness in broiler chicken diet containing jack bean seed (*Canavalia ensiformis*) with different protein level toward internal organ size

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Abstract. This study was conducted with the intention of evaluating the use of the protease enzyme in broiler feed containing jack bean seed (*Canavalia ensiformis*) with the different levels of protein towards the internal organs of broiler chicken starter. Bird samples used were 24 broilers of the male lohmann strain taken from 120 chickens. The study design used was a factorial completely randomized design (FCRD) with 2 factors treatment and 3 replications (each replication used 2 samples of bird). The first factor was the level of protein (CP 22% and CP 19.5%) and the second factor was the use of enzymes (non-protease and protease). Data were analyzed by using analysis of variance (ANOVA). The result showed that the use of low protein levels in broiler feed containing jack bean seed significantly (P <0.05) increased the percentage of pancreatic weights but could be improved by the use of protease enzymes. Levels of protein treatment did not significantly affect the percentage of heart, liver and bile weights. The use of protease enzymes significantly (P <0.05) decreased the percentage of pancreatic and bile weights but did not significantly affect the percentage of liver and heart weights.

1. Introduction

The demand for broiler chicken meat in Indonesia is increasing every year. [1] reported that consumption of broiler chicken meat in 2015 to 2016 increased from 4797 kg/capita to 5110 kg/capita. Broilers are in great demand because of their high productivity and for their maintenance in only a short time. The nutritional content of carcasses is also quite good, its protein content reaches 22.15% - 23.33% with fat content 1.62% - 2.27% [2]. The good performance especially body weight growth in broilers must be supported with a good feed quality.

Nowadays, Broiler chicken feed in Indonesia uses corn as an energy source and soybean meal as a source of protein. The fulfillment of soybean meal is still difficult to conduct so the fulfillment must be done by import. According [3] in 2016, Indonesia imported soybean meal of 3 million tons in 1994. It is necessary to explore the protein feed ingredients to reduce the use of soybean meal in broiler chicken feed. One of these feed ingredients is the use of jack bean seed (*Canavalia ensiformis*). Jack bean seed has dry matter 86.5% - 96.2%, energy 351.3 kcal /100 g - 456.5 kcal /100 g, total starch 24.7 - 36.9%, crude protein 22.8 - 35.3%, ether extract 1.6% - 12.1%, crude fiber 4.7 - 11.4%, and ash...
content 2.3-5.8%. According to [4] the use of jack bean seed can increase the metabolic energy of broiler chicken starter.

The use of Jack bean seed as a protein source of feed has a weakness in the form of an anti-nutrient trypsin inhibitor of 7.96 mg / g of material even though it has been processed by heating (the seed by) using an autoclave [4]. The use of 7% of Jack Bean seed in broiler chicken starter feed can reduce body weight but can be improved by the addition of a protease enzyme [4]. The use of the protease enzyme is one of the solutions to improve the quality of the Jack bean seed.

Protease enzyme is one of the enzymes that can be used to improve feed efficiency in broilers. Protease enzymes are able to catalyze the breakdown of peptide bonds in polypeptides and proteins through hydrolysis reactions into simpler molecules like short chain peptides and amino acids [5]. Increased protein digestibility due to the use of protease enzymes in feed can be used to reduce feed costs by reducing the value of protein in the feed formulation. According to [4] the body weight reduction of broiler chickens fed with low protein level (19.5%) can be improve again to match the body weight of broiler chickens fed according to their standard protein level (22%) with supplementation of protease enzymes by 12500 units per kg of feed ration. The use of protease enzymes is very effective to be used in broiler starter feed containing Jack bean seed (Canavalia ensiformis) because it can improve the performance of broiler chickens to match the basal feed by using soybean meal [4].

The Evaluation of an enzyme (quality) is not only done by measuring its effectiveness in improving animal performance. Animal health is one of the ways that also needs attention. One of the ways that can be done to determine the effectiveness of the protease enzyme as additional feed for broiler chicken is to measure the response of organ size in broilers. Normally, the size of the organ that has no enlargement or swelling indicates that the animal is in healthy condition or their internal organs do not work hard during the metabolic process. This study aimed to evaluate the use of the protease enzyme in broiler feed containing Jack bean seed (Canavalia ensiformis) with different levels of protein towards internal organs of broiler chicken starter.

2. Methods
2.1 Material
2.1.1 Animal
Bird samples used in this study were 24 broiler chicken taken from 120 broilers which were previously reared since Day Old Chick (DOC). The broiler chicken used is male broiler chicken strain Lohhman MB 202 PT Japfa Indonesia.

2.1.2 Protease Enzyme
The protease enzyme used in this study from [6] with a row material in the form of carrier (CaCO₃, and wheat flour), and silicon dioxide. The brand name for the protease enzyme used was concentrate-P. The protease activity was 12500 hemoglobin units (HUT) / kg diet. The use of this protease enzyme was 0.5 g / kg of diet. This enzyme was extracted from Bacillus licheniformis.

2.1.3 Diet
The diet used during the DOC phase up to age 7 days were commercial feed. Furthermore, broiler chickens were given a treatment of feed containing Jack bean seed (Canavalia ensiformis) which was formulated based on recommendation [7]. The composition of feed ingredients and chicken feed for broiler chicken started is presented in Table 1 below.

| FEED MATERIAL (%) | P1E0  | P2E0  | P1E1  | P2E1  |
|--------------------|-------|-------|-------|-------|
| Yellow Corn        | 48.50 | 52.85 | 48.50 | 52.85 |
| Rice Bran          | 8.50  | 9.00  | 8.50  | 9.00  |
| Crude Palm Oil     | 2.20  | 2.20  | 2.20  | 2.20  |
### Nutritional Composition

| Component                  | P1E0  | P2E0  | P1E1  | P2E1  |
|----------------------------|-------|-------|-------|-------|
| Corn Gluten Meal           | 7.00  | 3.00  | 7.00  | 3.00  |
| Soybean Meal               | 23.70 | 22.80 | 23.70 | 22.80 |
| Jack Bean Seed             | 7.00  | 7.00  | 7.00  | 7.00  |
| Premix                     | 0.50  | 0.50  | 0.50  | 0.50  |
| Calcium Carbonat           | 0.40  | 0.40  | 0.40  | 0.40  |
| Dicalcium Phosphate        | 1.50  | 1.50  | 1.50  | 1.50  |
| L-lysine                   | 0.50  | 0.50  | 0.50  | 0.50  |
| DL-Methionine              | 0.20  | 0.25  | 0.20  | 0.25  |
| **Total**                  | 100   | 100   | 100   | 100   |
| **Protease Enzyme (g/kg)** | 0     | 0     | 0.5   | 0.5   |

### Nutrient Analysis

| Nutrient                  | P1E0  | P2E0  | P1E1  | P2E1  |
|---------------------------|-------|-------|-------|-------|
| Dry Matter (%)            | 98.80 | 99.15 | 98.80 | 99.15 |
| Ash Content (%)           | 5.28  | 5.29  | 5.28  | 5.29  |
| Crude Protein (%)         | 22.02 | 19.57 | 22.02 | 19.57 |
| Crude Fat (%)             | 6.53  | 6.67  | 6.53  | 6.67  |
| Crude Fiber (%)           | 4.58  | 4.69  | 4.58  | 4.69  |
| Metabolizable Energy (Kkal/kg) | 3095.07 | 3091.17 | 3095.07 | 3091.17 |
| Calcium (%)               | 1.04  | 1.06  | 1.04  | 1.06  |
| Phosphor Total (%)        | 0.57  | 0.56  | 0.57  | 0.56  |
| Phosphor Available (%)    | 0.41  | 0.40  | 0.41  | 0.40  |
| Lysine (%)                | 1.33  | 1.28  | 1.33  | 1.28  |
| Methionine (%)            | 0.56  | 0.56  | 0.56  | 0.56  |
| Protease Enzyme (HUT/KG)  | 0     | 0     | 12500 | 12500 |

P1E0 = feed containing jack bean seed, crude protein 22%, without protease treatment, P2E0 = Diet containing jack bean seed, crude protein 19.5%, without protease treatment, P1E1 = Feed containing jack bean seed, crude protein 22%, protease treatment, P2E1 = Feed containing jack bean seed, crude protein 19.5%, protease treatment.

### 2.2 Procedure

#### 2.2.1 Measurement of Internal Organs

Organs in broilers were measured after slaughtering broilers. The internal organs were separated from the body of broiler chickens after being slaughtered. The internal organs measured were the liver, pancreas, bile, and heart. The weight of the internal organ and percentages it with the live weight of broiler chickens before being slaughtered. The formula for calculating the weight percentage of broiler chicken organs is:

$$\text{Percentage Organ weight (\%)} = \frac{\text{Organ weight (g)}}{\text{Body weight (g)}} \times 100\%$$

#### 2.2.2 Experiment Design and Data Analysis

The experimental design used in this study was Factorial Completely Randomized Design (FCRD). The treatment factors in this study were broiler chicken (feed) ration containing Jack bean seed (*Canavalia ensifromis*) with different crude protein levels (22% and 19.5%) and the use of enzymes (non-enzymes and protease). Data analysis used was Analysis of Variance (ANOVA), The mathematical model used was based on [8] :

$$Y_{ijk} = \mu + A_i + B_j + AB_{ij} + \epsilon_{ijk}$$

$X_{ij}$: Observation values that obtain a combination of $i$ level protein treatment, and $j$ protease enzyme.
µ : means
Ai : The effect of i level protein treatment
Bj : The Effect of i protease enzyme treatment
ABij : The interaction effect of treatments of i protein level and j protease enzyme
Eijk : Error of this treatment

2.2.3 Treatment

The treatments used in this study were:
P1E0 : Broiler chicken feed ration containing Jack Bean seed, CP 22%, without the protease enzyme.
P2E0 : Broiler chicken ration feed containing Jack Bean seed, CP 19.5%, without the protease enzyme.
P1E1 : Broiler chickens ration feed containing Jack Bean feed, CP 22%, protease enzyme supplementation.
P2E1 : Broiler chickens feed containing Jack bean seed, CP 19.5%, protease enzyme supplementation.

3. Results and discussion

The use of a low protein level in the chicken feed containing Jack bean seed (*Canavalia ensiformis*) significantly (P <0.05) increased the percentage of pancreas weights but could be improved by the use of the enzyme protease. Protein treatment did not significantly affect the percentage of heart, liver and gall bladder weight. The use of protease enzymes significantly (P <0.05) decreased the percentage of pancreatic and gall bladder weights but did not significantly affect the percentage of liver and heart weights. There was no interaction between differences in protein levels and the use of protease enzymes in broiler chicken diet containing Jack bean seed. Internal organ data (of) broiler chickens that were gave feed containing Jack bean seed (*Canavalia ensiformis*) with different levels of protein and the addition of the protease enzyme shown in Table 2.

**Table 2.** Internal organ of broiler chickens with Jack bean seed (*Canavalia ensiformis*) with different protein levels and addition of protease enzymes.

| Protein Treatment | Enzyme | Average |
|-------------------|--------|---------|
|                   | E0     | E1      |         |
| Liver (%)         |        |         |         |
| P1                | 2.89±0.16 | 2.79±0.01 | 2.84 ± 0.07 |
| P2                | 2.91±0.04 | 2.87±0.18 | 2.89 ± 0.03 |
| Average           | 2.90±0.01 | 2.83±0.05 |         |
| Gall Bladder (%)  |        |         |         |
| P1                | 0.07±0.01 | 0.05±0.02 | 0.06 ± 0.02 |
| P2                | 0.07 ± 0.01 | 0.05 ± 0.01 | 0.06 ± 0.01 |
| Average           | 0.07 ± 0.01b | 0.05 ± 0.01a |         |
| Pancreas (%)      |        |         |         |
| P1                | 0.34 ± 0.04 | 0.30 ± 0.04 | 0.32±0.04a |
| P2                | 0.43 ± 0.02 | 0.34 ± 0.02 | 0.39±0.05b |
| Average           | 0.38 ± 0.06b | 0.32±0.04a |         |
| Heart (%)         |        |         |         |
| P1                | 0.57 ± 0.06 | 0.44 ± 0.04 | 0.50±0.09 |
| P2                | 0.60±0.17  | 0.53±0.05  | 0.57±0.07 |
| Average           | 0.59±0.02  | 0.49±0.07  |         |
P1 = feed with crude protein 22%, P2 = feed with crude protein 22%, E0 = Non enzyme treatment, E1 = Enzyme protease treatment. Different letters in the same table or column show significantly different values (P <0.05).

3.1 Liver
The treatment of protease enzymes in broiler chicken feed containing Jack bean seed (*Canavalia ensiformis*) with different levels of protein had no effect on liver weight. The protease enzyme did not cause toxicity in broiler chickens. Protease enzymes helped protein digestion. The process of degradation of proteins into simpler compounds was faster with the help of the protease enzyme, so proteins were more easily absorbed in the digestive tract. The protease enzyme used did not contain compounds that endangered the health of livestock so it did not affect the performance of the liver and therefore the size of the liver did not change significantly. The function of the liver is to neutralize toxins found in the body [9].

The size of the liver in this study was 2.79% - 2.91%. The liver was slightly larger than normal size according to [10] which was 1.7% to 2.8%. This is presumably due to the use of jack bean seed in the ration because jack bean seed contain trypsin inhibitors of 7.9 mg / 100 g [4]. Besides trypsin inhibitors jack bean seed contain several other antinutrients, namely choline, hidrozianine acid, troginelin and antichimotripsin, phenols, isoflavones, niacin, saponins [11].

3.2 Gall Bladder
The use of protease enzymes in broiler chicken feed containing jack bean seed (*Canavalia ensiformis*) with different protein levels could reduce the weight of gall bladder. The low weight percentage of gall bladder indicates that bile does not work hard to help the process of digestion and metabolism of nutrients. The weight and volume of bile is influenced by the type of feed, nutritional status, and enterohepatic biliary circulation [12]. Protease enzymes (helped) the process of digestion and absorption of protein so the protein was more easily absorbed by the body. The nutritional status of feed, especially crude protein can be improved by the provision of a protease enzyme. Bile functions to process fat absorption and excretion of toxins from metabolic waste [12]. Gall bladder consists of bile salts, cholesterol, pigments, fats, organic salts and lecithin [13].

The percentage of bile weight in this study ranged from 0.05% - 0.07%. This percentage does not differ greatly from the research of [14] which is 0.029% - 0.097%. Normally the size of this bile indicates that broiler chickens do not experience any disturbance in the metabolic system in the body.

3.3 Pancreas
Reduction of protein level in chicken feed to 19.5% broiler chickens containing Jack bean seed (*Canavalia ensiformis*) have an impact on increasing the size of the pancreas but could be repaired again by the addition of protease enzymes so it produced the same value with the treatment by using 22% PK level. Protein deficiency in poultry would have a negative impact on the body's metabolic system. The deficiency of protein in the feed was thought to stimulate the work of the pancreas to produce digestive enzymes harder so the protein could be used efficiently. Secretion of pancreatic enzyme was stimulated by the hormone cholecystokinin. Cholecystokinin is a hormone secreted by the small intestinal mucosa which functions to stimulate the secretion of the gallbladder [15]. The pancreas secretes pancreatic fluid to digest starch, fat and protein. The pancreas has two functions, namely exocrine and endocrine. Exocrine functions to supply digestive enzymes while endocrine functions to regulate nutrients to be absorbed in the body [9]. Protease releases from the pancreas and it is controlled by the hormone cholecystokinin produced by intestinal mucosal cells [16].

The use of protease enzymes in broiler diets feed containing jack bean seed (*Canavalia ensiformis*) with different levels of protein could reduce the percentage weight of the pancreas. Supplementation of the protease enzyme in the feed would help the digestive process. Protease enzymes are able to catalyze the breakdown of peptide bonds in polypeptides and proteins through hydrolysis reactions into simpler molecules such as short chain peptides and amino acids [5]. It is suspected that the pancreas did not need too much production of protease enzymes in the body if the protease enzyme has been added to the food so it worked lighter. The percentage weight of the organ...
of the pancreas did not increase if the pancreas did not work too hard. Increased pancreatic weights is a form of adaptation to meet the increased needs of digestive enzymes [17].

The value of the percentage of pancreatic weights in this study was 0.30% - 0.43%. The weight of the pancreas was within the normal range according to [18], i.e. 0.25% -0.40% of body weight. Normally the weight of the pancreas indicated that the body's metabolic system in terms of secretion of digestive enzymes was operated normally.

3.4 Heart

The use of protease enzymes in feed containing Jack bean seed (Canavalia ensiformis) did not affect the percentage of heart weight. The heart is an organ that functions as a pump in blood circulation in the body. The heart is very susceptible to poisons and anti-nutrients, heart enlargement can occur if there is accumulation of toxins in the heart muscle [19]. The value of heart weight percentage in this study was 0.44% - 0.60%. This value was included in the value of the percentage of normal heart weight according to [20] which is 0.5% -1.42%. This indicated that the treatment of protease enzymes and different protein levels in broiler chicken feed containing Jack bean seed (Canavalia ensiformis) did not interfere with the function of the heart.

4. Conclusion

The use of broiler chicken starter feed containing jack bean (Canavalia ensiformis) with a low protein level (19.5%) could increase the percentage of the weight of the pancreas broiler chicken but could be improved by supplementation of protease enzyme. The use of protease enzymes in feed containing Jack bean seed (Canavalia ensiformis) could improve the organ size respond in broilers.

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