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Effect of protease in diets containing winged bean seeds (*Psophocarpus tetragonolobus*) on growth performance of broiler chickens

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Abstract. This study aims to determine the effect of adding protease enzyme in diets that contains different levels of winged bean seeds on the growth performance of broiler chickens. In this study, a total of 240 one-day-old chicks were randomly grouped within six treatments and four replicates in a 3×2 factorial arrangements. The first factor was the level of winged bean seeds at 0%, 2.5%, and 5%, while the second was the enzyme treatment with and without protease. The results showed that the additive protease interaction and level of winged bean seeds on the diet had a significant effect on the feed conversion ratio value ($P<0.05$). Meanwhile, on the feed intake, final, and body weight gain had no significant effect ($p>0.05$). Furthermore, the protease treatment significantly decreased feed intake in 35-day old broilers. ($P<0.05$). Based on these results, the addition of protease enzyme at the levels has the potential to offset the effect of winged bean seeds on growth performance of broiler chickens.

Keywords: Local raw materials, growth performance, protease, protein source.

1. Introduction

The need for protein increases every year due to population growth and public awareness of a balanced diet. Therefore, broiler chickens are one of the livestock that is widely consumed by people to meet this need. These chickens have tender meat, relatively cheap prices, and a short life cycle. However, one important factor that affects the production and quality of the meat is feed. Feed is one of the important factors for the success of a livestock business, which is 65%-75% of the total production cost [1]. Soybean meal is among the main raw materials of broiler chicken feed as a source of protein and the suppliers are still imported. In 2018, Indonesia imported 2,585,809 tons of soybeans [2], hence, the use of local protein sources such as winged bean (*Psophocarpus tetragonolobus*) as an alternative is required.

Moreover, the winged bean is a legume species with high protein content in the seeds that grows well in tropical areas. The production of winged beans is 35.50–40 t young pods/ha or the equivalent of 4.50 t of dry seeds/ha [3]. These legumes are a duplicate of soybeans in terms of nutritional and composition value of the seeds. Both of them contain similar proteins, oils, vitamins, minerals and essential amino acids [4]. Furthermore, winged bean seeds contain crude protein of approximately 28.4–29.2% [5], however, its use in the diet is still rare.

The winged bean seeds contain anti-nutritional substances such as protease inhibitors, which consist of chymotrypsin and trypsin [6]. These substances inhibit the function of the trypsin enzyme, which



reduces the rate of protein absorption. One of the methods that are used to reduce protease inhibitor activity is heating, which also improves the nutritional quality of the legumes. The seeds heated by autoclave for 90 minutes to reduce the trypsin inhibitor content from 16.92 to 2.08 mg/g and increase protein digestibility in vitro [7]. Another weakness of winged bean seeds is lower digestibility than soybean meal. Therefore, a method such as the addition of enzymes is needed to improve the digestibility of the feed ingredients. The enzyme is a protein that has biochemical activity as a catalyst for a reaction. Meanwhile, protease is the type of enzyme that increases the digestibility of amino acids from feed ingredients [8]. Proteases have been widely used to improve the quality of feed ingredients [9] and crude protein diets [10]. Therefore, this study aims to determine the effect of adding protease enzyme to the diet containing different levels of winged bean seeds on the growth performance of 35-day old broilers.

2. Materials and methods

2.1. Enzyme characteristics

The protease (Concentrase-P) used in this study was an exogenous commercial protease enzyme in powder form, which was extracted from *Bacillus licheniformis*. Other ingredients of this enzyme were wheat flour, lime, and silicon dioxide. The dose of the protease enzyme used was 0.5 g/kg of feed with a concentration of 12,500 units/kg.

2.2. Winged bean seed

The ground-winged bean seeds were heated by autoclave at 120 °C for 90 minutes. These beans were cooled to room temperature before being mashed and sieved with a 0.1 mm sieve shaker [7].

2.3. Experimental design

A total of 240 day-old broiler chicks (Cobb 500) used in this study were divided into a 3x2 factorial arrangement with six treatments and four replicates of 10 chicks each. Broiler chickens were reared for 35 days, with days 1–7 as the pre-starter, 8–21 days as the starter, and 22–35 days as the finisher phase. The treatments consisted of 0% winged bean seeds with and without protease enzyme, 2.5% winged bean seeds with and without protease enzyme, 5% winged bean seeds with and without protease enzyme. Furthermore, the treatment was administered on day 14 of maintenance, which was previously being given adapted feed. The feed and drinking water were given ad libitum. The starter and finished diets were prepared based on SNI.8173.2.2015 [11] and SNI 8173.3.2015 [12], respectively. The ingredients and nutrition compositions of the experimental diets are shown in Table 1. Furthermore, final body weight, body weight gain, feed intake, and feed conversion ratio were calculated.

2.4. Statistical analysis

To determine the effect of treatment, the data were analyzed using analysis of variance (ANOVA). Duncan's multiple range test was used when the variables showed a significant result, and the statistical procedures were computed using SPSS version 20.

Table 1. Ingredients and nutrition compositions of experimental diets.

Diet Ingredients (%)	Starter phase			Finisher phase		
	K0	K1	K2	K1	K1	K2
Corn	55,00	55,00	55,00	58,80	58,80	58,80
Soybean meal	26,50	25,00	23,20	22,50	21,55	19,50
Winged bean seeds	0,00	2,50	5,00	0,00	2,50	5,00
Corn gluten meal	7,00	7,00	7,00	8,00	8,00	8,00
Crude palm oil	2,10	2,10	2,10	2,50	2,50	2,50
Rice bran	6,50	5,50	4,80	5,30	4,00	3,30
Calcium carbonate	0,50	0,50	0,50	0,50	0,50	0,50
Dicalcium Phosphate	1,50	1,50	1,50	1,50	1,50	1,50
Premix	0,50	0,50	0,50	0,50	0,50	0,50
L-lysine	0,20	0,20	0,20	0,20	0,20	0,20
DL-methionine	0,20	0,20	0,20	0,20	0,20	0,20
Total	100	100	100	100	100	100
Nutrition compositions						
Dry matter (%)	86,16	86,22	86,28	86,00	86,06	86,12
Crude protein (%)	21,81	21,89	21,97	20,72	20,64	20,52
Ether extract (%)	6,74	7,15	7,56	6,83	7,27	7,70
Crude fiber (%)	2,70	2,66	2,62	2,46	2,64	2,65
Metabolisable energy (Kkal kg ⁻¹)	3112	3115	3117	3161	3160	3159
Calcium (%)	1,10	1,09	1,09	0,98	0,98	0,98
Phosphorus (%)	0,60	0,62	0,61	0,59	0,57	0,56
Lysine (%)	1,52	1,59	1,67	1,42	1,48	1,53
Methionine (%)	0,86	0,86	0,86	0,84	0,84	0,84

K0: Diet with winged bean seeds 0%, K1: Diet with winged bean seeds 2.5%, K2: Diet with winged bean seeds 5%.

3. Result and discussion

The effect of interaction adding protease enzyme to diets containing different levels of winged bean seeds on the growth performance of broiler chickens aged 35 days is shown in Table 2. The results showed that there was no significant effect of adding protease enzyme and winged bean seeds level on final and body weight gain ($p > 0.05$). However, the addition of protease to the diets with concentrations of 2.5% and 5% winged bean seeds had better final and body weight gain than without addition, although it was not statistically different. These results are in line with previous studies, which were also insignificant, but had a higher final and body weight gain on the diets with supplemented protease enzyme, and also on low protein diets [10], [13]. This occurred because the use of proteases in diets increases protein digestibility value. Therefore, the protein from winged bean seeds is optimally used, even at poor quality. A previous study had shown that increased digestibility of amino acids in broiler chickens improves growth performance and intestinal health [14], and reduces the negative effects of trypsin inhibitor [15]. Similarly, the use of protease increases trypsin activity in the pancreas [16].

The analysis of variance results showed that the interaction of addition protease enzymes to diets containing different levels of winged bean seeds had no significant effect on the feed intake ($p > 0.05$). But, protease enzyme treatment had a significant effect ($p < 0.05$). Also, treatment without protease had a higher intake due to the tendency of consuming more feed to fulfil their protein needs for growth. Meanwhile, the decrease in feed consumption in the treatment with the protease enzyme occurred due to its ability. Hence, the released dietary protein is easily absorbed and the digestibility is also increased

for the protein from winged bean seeds to be optimally used by livestock. The addition of protease enzymes reduces feed consumption by 42.69 g. This is in line with previous studies, which stated that the addition of protease enzymes to the diets reduces feed consumption, even in low-protein diets [10], [17], [18].

Table 2. Effect of levels of winged bean seeds and protease enzymes on growth performance in 35-day old broiler chickens.

Items	Diets	Protease		Average
		Without	With	
Final body weight (g)	K0	808.33 ± 8.50	832.46 ± 38.37	820.39 ± 28.78
	K1	807.74 ± 29.23	818.15 ± 22.02	812.95 ± 24.60
	K2	799.81 ± 5.27	810.14 ± 8.28	804.97 ± 8.48
Average		805.29 ± 16.64	820.25 ± 25.41	
Body weight gain (g)	K0	764.96 ± 8.79	788.97 ± 36.94	776.96 ± 27.98
	K1	762.68 ± 28.75	773.94 ± 21.03	768.31 ± 24.08
	K2	753.75 ± 3.70	766.25 ± 8.08	760.00 ± 8.85
Average		760.46 ± 16.61	776.39 ± 24.65	
Feed intake (g)	K0	1422.44 ± 9.71	1372.76 ± 63.32	1397.60 ± 49.64
	K1	1427.87 ± 51.18	1387.20 ± 24.83	1407.54 ± 43.12
	K2	1435.40 ± 10.28	1397.67 ± 14.29	1416.53 ± 23.23
Average		1428.57 ± 28.28 ^b	1385.88 ± 37.83 ^a	
Feed conversion ratio	K0	1.86 ± 0.02 ^c	1.74 ± 0.02 ^a	1.80 ± 0.07 ^a
	K1	1.87 ± 0.01 ^c	1.79 ± 0.02 ^b	1.83 ± 0.04 ^b
	K2	1.90 ± 0.01 ^d	1.82 ± 0.01 ^b	1.86 ± 0.04 ^c
Average		1.88 ± 0.02 ^b	1.79 ± 0.04 ^a	

^{a,b} Means not sharing a common superscript in a same of column-subgroup are significantly different ($p < 0.05$)
K0: diet with winged bean seeds 0%, K1: diet with winged bean seeds 2.5%, K2: diet with winged bean seeds 5%.

The interaction of protease enzyme to diets containing different levels of winged bean seeds have a significant effect on the value of feed conversion ratio (FCR) ($p < 0.05$). The diets containing winged bean seeds with protease enzyme have a smaller FCR value than those without protease enzyme. Meanwhile, the lower winged bean seeds in the diet are directly proportional to the FCR value. The treatment with 5% winged bean seeds with the enzyme has a smaller FCR value than treatment with 0% of the seeds without protease enzyme. This FCR value is related to body weight gain and feed intake of broiler chickens. The FCR value is an indicator of the quality of the diets used, therefore, a smaller value indicated that a better quality of feed [19]. Due to the lower feed intake and higher body weight produced, the feed intake is optimally used by livestock for growth. The decrease in FCR value with protease enzymes in poultry diets has been previously reported. In the previous study, a 4% reduction in broilers was achieved when the diet was supplemented with an exogenous protease [20] and a protease enzyme purified from *Bhacillus licheniformis* [21].

Generally, broilers fed on a poor quality protein diet, gained less weight, consumed more feed, and had a worse feed conversion ratio. Hence, a significant reduction in feed intake and improvement in conversion ratio were observed due to protease supplementation.

4. Conclusion

The results showed that the addition of protease enzyme at the levels has the potential to offset the effect of winged bean seeds on growth performance of broiler chickens aged 35 days. Meanwhile, the use of

winged bean seeds up to 5% has no significant effect on the final and body weight gain. Therefore, the addition of protease enzyme in a diet containing winged bean seeds reduces the feed conversion ratio in broiler chickens.

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