Visceral organ weight of broiler chicken fed different level protein and protease enzyme supplementation diet

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Abstract. The aim of this study was to evaluate the effects of different levels of protein and protease enzyme supplementation on the visceral organ weight of broiler chickens. A total of 24 day-old Lohmann broiler chicks were taken from 120 chickens which have been reared previously since the day old chicks. The experiments were conducted in 2x2 factorial design, with two treatments and three replication, to determine the effects of adding protease enzyme (0 HUT/kg and 12500 HUT/kg) and different crude protein levels (19.5% and 22%) on the visceral organs weight (liver, gall bladder, pancreas, and heart). Data were analyzed using analysis of variance (ANOVA) and continued with the Duncan multiple range test. Results showed that all the treatments whether protein levels or enzyme addition did not affect significantly the organs weight. Further, there was no significant interaction between protein levels and enzyme addition in all organs weight except the pancreas weight has shown a significant interaction (P<0.01) between the protein levels and enzyme addition. Although the levels of the crude protein could influence the broiler performance as well as the visceral organs weight, protease addition has alleviated the negative effects of protein reduction on the visceral organ weight. Therefore, in conclusion, protease addition can be used safely with low protein levels without any adverse effects on the visceral organ weight of broiler chickens.

1. Introduction

During the last four decades, the poultry sectors have shown a remarkable growth all over the world [1] such a huge success in the poultry farming business is depend mainly on the feed and feeding quality. According to Thirumalaisamy et al (2019) the feed costs represent about 60–70% of the total production costs of the modern broiler practices [2]. However, protein is considered as the second most substantial nutrient and the most expensive components in poultry nutrition [2,3]. Therefore, reducing the dietary crude protein has become public interest becauseit is considered as an important factor for eliminating the total costs of meat production and the environmental loads that caused by the industries of the livestock [1].

In addition, more than 3% of the previous workhave reported that the reduction of dietary crude protein is responsible for reducing broiler performance [1]. In contrast, the reducing of the dietary crude proteinis mentioned to be beneficial as the protein metabolism is associated with higher heat increment
than that of carbohydrates and lipids [4]. In the same time, the reduction of dietary CP and ME may affect negatively feed intake and the growth rate [5].

On the other hand, supplementation with enzymes is used to eliminate the anti-nutritive components and to enhance the nutrient utilization which affects the broiler performance [6]. Thus several kinds of exogenous enzymes in the poultry nutrition, including the non-starch polysaccharide (NSP) degrading enzymes, are commonly used [7]. Since the protein digestion in monogastric animals is derived mainly by the endogenous proteases throughout two stages of gastric digestion, in an acidic environment, and the pancreatic digestion in the small intestine [4]. Protease is mainly used to improve the nutrient utilization because not all the protein components are completely utilized by chickens [3]. However, the effects of different protein levels and exogenous protease addition on growth performance and the nutrient digestibility in broilers have been studied by Mahmood (2017) who concluded that the addition of exogenous protease enzyme has improved the feed intake and the body gain at any age, while feed intake and the body gain have been reduced by decreasing the protein levels [8]. The effects of protease supplementation and different crude protein levels are not yet fully demonstrated, therefore the aim of this study was to evaluate the effects of different protein levels and protease supplementation on the visceral organs of broiler chickens.

2. Method

2.1. Experimental design and diets
In this study, a commercial diet, which obtained from PT. Charoen Phokphand, were used in pre-starter phase. The experiments were done from day 8 to achieve the desired performance because the composition of pre-starter diet can influence the subsequent growth and the development of broiler chickens [9]. Therefore, at the beginning of the experiments from 1–7 days, birds were fed pre-starter which contain equal levels of protein. The experimental diets were given since the starter phase at the age of 8 days. The requirement of crude protein for broiler chicken in the 8–25 day old age is 21.5 (%) [10]. Experimental diets were formulated and presented in table 1. The chemical compositions of experimental diets are shown in the (table 2). The chemical compositions of experimental diets are shown in the (table 2). The ingredients have been mixed and pelleted at 65 °C. The enzyme was coated using (CaCO3, wheat flour, and silicon dioxide) to avoid the negative impacts of excessive heat. The experimental diets were arranged in 2x2 factorial randomized completely design with two factors protein levels (19.5% and 22% CP) and protease addition (0 HUT/kg and 12500 HUT/kg).

Three replication for each pen were used and the treatments were arranged as follow:
P1E1= basal diet level of protein (22%) + protease supplementation (0 HUT/kg)
P1E2= basal diet level of protein (22%) + protease supplementation (12,500 HUT/kg)
P2E1= basal diet level of protein (19.5%) + protease supplementation (0 HUT/kg)
P2E2= basal diet level of protein (19.5%) + protease supplementation (12,500 HUT/kg).

2.2. Enzyme composition.
A purified microbial protease (Concentrate-P; Canadian Bio-Systems Inc., Calgary, Alberta, Canada) was used in this study. This enzyme derived from Bacillus licheniformis. The protease activity was 12,500 Haemoglobin units (HUT)/kg. The manufacturers recommended inclusion rate is 0.5 g/kg of feed. Other ingredients contained in the protease enzyme used are carrier (CaCO3, and wheat flour), and silicon dioxide.
Table 1. Feed formula of a basal diet, as-fed basis starter phase.

| Ingredient     | P1E1 | P1E2 | P2E1 | P2E2 |
|----------------|------|------|------|------|
| Corn (%)       | 50.5 | 50.5 | 50.5 | 50.5 |
| Rice bran (%)  | 10.4 | 10.4 | 10.4 | 10.4 |
| SBM (%)        | 25.9 | 25.9 | 25.9 | 25.9 |
| CGM (%)        | 8    | 8    | 3.2  | 0.3  |
| CPO (%)        | 2.2  | 2.2  | 2.4  | 2.4  |
| CaCO₃ (%)      | 0.6  | 0.6  | 0.3  | 1.3  |
| DCP (%)        | 1.2  | 1.2  | 1.5  | 0.5  |
| Premix (%)     | 0.5  | 0.5  | 0.5  | 0.5  |
| L-Lysin (%)    | 0.5  | 0.5  | 0.5  | 0.5  |
| Lysin (%)      | 0.2  | 0.2  | 0.25 | 0.25 |
| DL-Methionine (%) | 0 | 0.5 | 0 | 0.5 |
| Protease (HUT/kg of feed) | 0 | 0.5 | 0 | 0.5 |

P1E1= basal diet level of protein (22%) + protease supplementation (0 HUT/kg); P1E2= Basal diet level of protein (22%) + protease supplementation (12,500 HUT/kg); P2E1= basal diet level of protein (19.5%) + protease supplementation (0 HUT/kg); P2E2=basal diet level of protein (19.5%) + protease supplementation (12,500 HUT/kg).

Table 2. Chemical composition of a basal diet, as-fed basis starter phase.

| Nutrient                        | P1E1 | P1E2 | P2E1 | P2E2 |
|---------------------------------|------|------|------|------|
| Dry matter (%)                  | 98.84| 98.84| 99.13| 99.13|
| Ash (%)                         | 5.34 | 5.34 | 5.33 | 5.33 |
| Crude protein (%)               | 22.02| 22.02| 19.54| 19.54|
| Crude fat (%)                   | 4.69 | 4.69 | 4.82 | 4.82 |
| Crude fiber (%)                 | 4.23 | 4.23 | 4.31 | 4.31 |
| Metabolizable energy (kcal/kg)  | 3,094.35| 3,094.35| 3,091.13 | 3,091.13|
| Calcium (%)                     | 1.05 | 1.05 | 1.04 | 1.04 |
| Total phosphor (%)              | 0.55 | 0.55 | 0.59 | 0.59 |
| Phosphor available (%)          | 0.40 | 0.40 | 0.42 | 0.42 |
| Lysin (%)                       | 1.30 | 1.30 | 1.27 | 1.27 |
| Methionine (%)                  | 0.57 | 0.57 | 0.57 | 0.57 |
| Protease (HUT/kg of feed)       | 0    | 12,500 | 0    | 12,500 |

P1E1= basal diet level of protein (22%) + protease supplementation (0 HUT/kg); P1E2= Basal diet level of protein (22%) + protease supplementation (12,500 HUT/kg); P2E1= basal diet level of protein (19.5%) + protease supplementation (0 HUT/kg); P2E2=basal diet level of protein (19.5%) + protease supplementation (12,500 HUT/kg).

2.3. Birds and management
A total of day-old male Lohmann 120 broiler chicks were obtained from a commercial hatchery. Broiler chicks were randomly distributed to 12 floor pens (n=10 chicks/pen) with chaff that serve as deep litter material in a conventional open-sided poultry house and under the hot and humid tropical conditions. The temperature ranged from 23°C to 36°C, and the relative humidity was between 75% and 90%. Freely accessed water was provided ad libitum from day (d)1 to 25.
2.4 Sampling and measurements
At 25 d of age, 24 male chickens were randomly chosen from 120 chickens were weighed, brought to the slaughterhouse and cut according to the animal welfare law. Before slaughter, chickens were subjected to a total feed withdrawal of 8 h. After completing the slaughtering process, the digestive tract and internal organs such as heart, liver, pancreas and gall bladder were separated and weighted on 0.001 g digital scale. All the collected records were. The relative weight of each organ was calculated as follows: Relative weight = (organ weight/live body weight) x 100.

2.5 Data Analysis
The obtained data at the end of the experiment were subjected to the analysis of variance (ANOVA) using the statistical packages SPSS (version 22), and Duncan’s multiple range test, according to Steel and Torrie (1993) at confidence level (p=0.05), was used to compare among the treatments. The mathematic model based on [11]:

$$Y_{ijk} = \mu + A_i + B_j + A_iB_j + \varepsilon_{ijk}$$

$$X_{ij}$$ : Observation values that obtained a combination of the treatment of protein level to i, and protease enzyme to j
$$\mu$$ : General mean
$$A_i$$ : Effect of protein level treatment to-i
$$B_j$$ : Effect of protease supplementationtreatment to-j
$$A_iB_j$$ : Effect of treatment interactions on the level of protein to-i, and the enzyme protease to j
$$\varepsilon_{ijk}$$ :Error of treatment
$$i$$ :The number of treatment
$$j$$ :The number of replication

3. Results and discussion
The results of liver, pancreas, gall bladder, and the heart weight were presented in table 3. Result showed that all the treatments whether protein levels or protease supplementation did not affect significantly the visceral organ weight (P<0.05). However, there was no significant interaction between protein levels and protease addition on the all organs except the pancreas weight has shown a significant interaction between protein levels and protease supplementation.

3.1. Liver
The liver plays main important role in the digestion, metabolism, and regulating the broiler production. Among the dietary factors, the feed restriction, anti-nutritional factors, and the feed additives were reported to influence the structure and the metabolic function of the liver [12]. It has been demonstrated that the activities of liver enzymes decrease when the low-protein level is fed [13]. It was reported that the feed restriction, especially protein level, decease the weight of all organs in the starting and finisher phase [14]. The obtained results of the liver weight showed that all the treatments did not affect significantly the liver weight. According to Putnam (1991) the liver weight between 2.67% and 2.76% is considered in the normal range [15]. This indicates that the liver function did not affect by the protein levels because protease tends to improve the protein utilization.

3.2. Gall bladder
The gall bladder weighted 0.48–0.58% of the live weight. Faria et al (2019) found that gall bladder represents from 0.3 % to 1.15% of the broiler live weight [16]. Suprijatna et al (2005) was stated that the gall bladder weight is influenced by the nutritional status, type of feed, blood flow, and enterohepatic gall bladder circulation [17]. However, the main function of the gall bladder is the obsorbtion of the feed fat and the secretion of the wastes product. Addition of protease with different protein levels did not affect the gall bladder. The reduction of protein levels in the broiler diet corresponds with a reduction of the body weight due to the deficiency of protein content. Exogenous protease addition was reported
to improve the utilization of amino acids resulting in maintaining the broiler performance [18]. Therefore all the treatments did not affect the gall bladder weight.

**Table 3.** The effect of the diets with different protein level supplemented with protease on the internal organ size in broiler.

| Items       | Liver (%) | Heart (%) | Pancreas (%) | Gall bladder (%) |
|-------------|-----------|-----------|--------------|-----------------|
| CP (%)      |           |           |              |                 |
| 22 %        | 2.67      | .65       | .34          | .48             |
| 19.5 %      | 2.76      | .70       | .35          | .58             |
| P-value     | .553      | .469      | .602         | .359            |
| SE          | .095      | .047      | .013         | .007            |
| Protease (HUT/kg) |          |           |              |                 |
| 12500       | 2.64      | .65       | .32          | .47             |
| 0           | 2.79      | .70       | .36          | .60             |
| P-value     | .298      | .414      | .062         | .231            |
| SE          | .095      | .047      | .013         | .007            |
| INT         |           |           |              |                 |
| SE          | .135      | .066      | .018         | .010            |
| P-value     | .651      | .961      | .015         | .359            |

CP= Crude protein; 0 HUT/kg= without enzyme addition; 12500 HUT/kg= with enzyme addition; INT= interaction between protein and enzyme; SEM= standard error of mean, P-value at 0.05 confident level.

3.3. Pancreas
Pancreas is one of the most important organs in the digestive system. Pancreas was reported to produce the enzymes which hydrolyze all major nutrients into small monomers that can be absorbed into the blood or lymph [19,20]. Based on the data in table 3, results showed that the pancreas weight was 0.34–0.35% of the live weight at 19.5 and 22% CP, respectively, while the pancreas weight after enzyme addition has been changed from 0.36 to 0.32%. Frandson (1992) reported that enzyme addition has relatively decreased the pancreas weight [25]. Sturkie (1976) stated that pancreas normally represents 0.25-0.40% of the live weight [21]. Therefore the pancreas weight is still in the normal range. Similarly, it has been proposed that feeding broilers with diets which contain anti-nutritional factors could interfere with nutrient utilization resulting in an adversely affect including changes in the morphology and pathology of gut and organs which affect animal health and productivity [12]. The low-protein diet was suggested to result in a poor performance in broiler [22]. This reveals that the protease enzyme addition helps to improve the protein utilization and broiler performance [8] due to its ability to cleave the anti-nutritive component. Results also showed there was a significant interaction between the protein levels and protease supplementation. Ndazigaruye et al (2019) stated that the interaction could be positively or negatively between protease addition and protein levels depending on the age [23].

3.4. Heart
The percentage of heart weight which obtained in this study was 0.65% to 0.70%. The percentage weight was included in the normal range according to Nickle (1990) who states that the normal heart weight in broilers is 0.5%–1.42% of live weight [24]. Our finding implied that there was no significant effects on the heart weight because the main function of the heart is to circulate the blood in the body. These results supported the idea of the enlargement of the heart weight naturally occurred because of the accumulation of the toxic nutrient included in the feed [25].
4. Conclusion
In conclusion, the visceral organ weight did not affect, despite the negative impacts of reducing the dietary protein levels on body weight as well as the visceral organ weight. Therefore, protease can be used safely with low protein levels without any adverse effects on the visceral organ weight of broiler chickens.

References
[1] Kobayashi H, Nakashima K, Ishida A, Ashihara A and Katsumata M 2013 Effects of low protein diet and low protein diet supplemented with synthetic essential amino acids on meat quality of broiler chickens Anim. Sci. J. 84 489–95
[2] Thirumalaisamy G, Muralidharan J, Senthilkumar S, Sayee R, Hema and Priyadharsini M 2019 Cost effective feeding of poultry Int. J. Environ. Sci. Technol. 5 3997–4005
[3] Ndazigaruye G, Kim D H, Kang C W, Kang K R, Joo Y J, Lee S R and Lee K W 2019 Effects of low-protein diets and exogenous protease on growth performance, carcass traits, intestinal morphology, cecal volatile fatty acids and serum parameters in broilers Anim. 9 5–16
[4] Awad E A, Fadullah M, Zulkifli I, Farjam A S and Chwen L T 2014 Amino acids fortification of low-protein diet for broilers under tropical climate: ideal essential amino acids profile Ital. J. Anim. Sci. 13 270–74
[5] Law F L, Zulkifli I, Soleimani A F, Liang J B and Awad E A 2018 The effect of low-protein diets and protease supplementation on broilers chickens in a hot and humid tropical environment Asian-Austral. J. Anim. Sci. 3 1291–300
[6] Saleh A A, Duwood M M, Badawi N A, Ebeid T A, Amber K A and Azzam M M 2020 Effect of supplemental serine-protease from Bacillus licheniformis on growth performance and physiological change of broiler chickens J. Appl. Anim. Res. 48 86–92
[7] Gitoee A, Janmohammadi H, Taghizadeh A and Rafat S A 2015 Effects of a multi-enzyme on performance and carcass characteristics of broiler chickens fed corn-soybean meal basal diets with different metabolizable energy levels J. Appl. Anim. Res. 43 295–302
[8] Mahmood T, Mirza M A, Nawaz H and Shahid M 2017 Effect of different exogenous proteases on growth performance, nutrient digestibility, and carcass response in broiler chickens fed poultry by-product meal-based diets Livestock Sci. 200 71–5
[9] Ivanovich F V, Karlovich O A, Mahdavi R and Afanasyevich E I 2017. Nutrient density of prestarter diets from one to ten days of age affects intestinal morphometry, enzyme activity, serum indices and performance of Broiler Chickens.” Anim. Nutr. 3 258–65
[10] Leeson S, Summers J D 2005 Commercial Poultry Nutrition 3rd Ed (Nottingham: Nottingham University Press)
[11] Steel R G D dan Torrie J H 1993 Prinsip dan Prosedur Statistik (Pendekatan Biometrik) (Jakarta: Gramedia Pustaka Utama)
[12] Zaefarian F, Abdollahi M R, Cowieson A and Ravindran V 2019 Avian liver: The forgotten organ Anim. 9 1–23
[13] Hashemi S R, Ahzar, Maroufyan K, Elham L, and Teck H B M 2010 Change in growth performance and liver function enzymes of broiler chickens challenged with infectious bursal disease virus to dietary supplementation of methionine and threonine Am. J. Anim. Vet. Sci. 5 20–6
[14] Duarte C R A, Maria L M, Paulino V and Daniela F P 2014 Digestive enzymatic responses of chickens feed-restricted and refed as affected by age Egyptian Journal of Neurology, Psychiatry and Neurosurgery 51 289–96
[15] Putnam P A 1991 Handbook of Animal Science (San Diego: Academic Press)
[16] Faria N, Iriyanti N, Susanti E 2019 Efek penggunaan fermeherbafit enkapsulasi dalam pakan terhadap boobot dan persentase organ aksesoris ayam broiler; Effect of fermeherbafit encapsulation in broiler chickens feed on weight and percentage organs accessories J. Anim. Sci. Technol. 1 241–5
[17] Supriyata E, Atmomarsono U and Kartasudjana R 2005 Ilmu Dasar Ternak Unggas (Jakarta: Penebar Swadaya)
[18] Pilliang W G and Djojosoebagio S 2002 Fisiologi Nutrisi 4th Ed (Bogor: IPB Press)
[19] Murwani R 2010 Broiler Modern (Semarang: Widya Karya)
[20] Vertiprakhov V G, Grozina A A and Dolgorukova A M 2016 The activity of pancreatic enzymes on different stages of metabolism in broiler chicks Sel’skokhozyaistvennaya Biologiya 51 509–15
[21] Sturkie P D 1976 Avian Physiology 3th Ed (Berlin: Springer verlag)
[22] Mahmood T, Mirza M A, Nawaz H and Shahid M 2017 Effect of supplementing exogenous protease in low protein poultry by-product meal based diets on growth performance and nutrient digestibility in broilers Anim. Feed Sci. Technol. 228 23–31
[23] Ndazigaruye G, Kim D H, Kang C W, Joo Y J, Lee S R and Lee K W 2019 Effects of low-protein diets and exogenous protease on growth performance, carcass traits, intestinal morphology, cecal volatile fatty acids and serum parameters in broilers Anim. 9 2–16
[24] Nickle R, Schummer A and Seifrle E 1990 Anatomy of Domestic Bird (Berlin: Verlag Paul Parey)
[25] Frandson 1992 Anatomi dan Fisiologi Ternak (Gadjah Mada University Press: Yogyakarta)