Evaluation of crude cellulase from *Trichoderma viride* – fermented copra meal and its effect on feed digestibility and digestive organs development of broiler chickens

U Hatta¹, O Sjofjan², N Rugaya¹ and B Sundu¹

¹Animal Husbandry Department, University of Tadulako, Palu, Central Sulawesi, Indonesia
²Animal Science Department, University of Brawijaya, Malang, East Java, Indonesia

E-mail: ummianihatta71@gmail.com

Abstract. The study was conducted to examine the effects of supplementation of crude cellulase produced from *Trichoderma viride*–fermented copra meal (CM) on nutrient digestibility, apparent metabolizable energy (AME), digestive organ weight, protein, and cholesterol content of breast meat of broiler chickens. A total of 200 day-old male broiler chickens were used. The birds were fed with 5 different diets; T₁ = 0 g/kg CM, T₂ = 50 g/kg CM + crude enzymes, T₃ = 100 g/kg CM + crude enzymes, T₄ = 150 g/kg CM + crude enzymes and T₅ = 200 g/kg CM + crude enzymes. Feed digestibility, digestive organ weight, protein, and cholesterol content of breast meat were determined as parameters. On day 35, two birds from each replication were placed into metabolism cages for digestibility measurement. A completely randomized design was adopted with five treatments and five replications. The digestibility of protein, crude fiber, AME of the diets, and cholesterol content of the breast meat of broiler chickens fed the rations with crude enzyme addition were better than those of birds fed the T₁. The response of protein and crude fiber digestibility was curvilinearly over the increasing levels of CM in the diets and crude enzyme addition. Digestive organs weight, protein, and lipid percentages of the breast meat were not affected by the treatments. Supplementation of increasing CM diets with crude enzymes from *Trichoderma viride* produced higher protein, crude fiber digestibility, and AME of the diets and lower cholesterol content of breast meat.

1. Introduction
Research on using copra meal to increase its quality as a poultry feed ingredient has been the main concern in coconut producing countries [1,2]. An early study indicated that the supplementation of copra meal–based diets with lysine failed to improve broiler performance to the same body growth of broilers fed the corn-soy based diet [2]. The addition of lysine and methionine in the diets containing copra meal still produced lower body weight gain of broilers, compared to the broilers fed the corn-soy based diets [3]. The reasons for the failure to enhance the nutritive value of the amino acids–supplemented copra meal diets was due to inadequate nutrient intake as a result of high indigestible compounds present in copra meal. The high fiber content of copra meal led to this agricultural by-product bulkier and higher in water holding capacity [4] and longer passage time in the digestive tract [5].
Despite the fact that the use of enzymes in poultry feed to produce economic and environmental benefits, enzymes have mostly been used in cereal-based diets. The positive benefits of using commercial enzymes in poor quality diets have been proven in numerous publications, such as in palm kernel meal diet [6], copra meal diet [7], and rice bran based diet [8]. However, studies on the use of commercial enzymes in copra meal-based diets produced inconsistent results [7,9].

The limited success of using commercial enzymes in copra meal based diets is possibly related to the fact that commercial enzymes were not designed for corn-copra meal based diets, but mainly for corn-soy diets. Accordingly, specific enzyme product is needed when copra meal is used in high concentration in poultry diet. To produce this kind of enzyme, the use of copra meal as a substrate might improve the efficacy of the enzyme. Enzymes produced through solid-state fermentation technology were better than the conventional technology of submerged liquid fermentation [10]. Fungi, particularly in the species of Trichoderma, have been reported to be the potential to produce cellulase [11,12]. The current study was carried out to determine the effect of the crude enzyme from Trichoderma viride-fermented CM on digestibilities of the diet, digestive organs weight, protein, and cholesterol contents of breast meat of broiler chickens.

2. Materials and Methods

2.1. Fermentation of copra meal (CM) and crude enzyme production
A fungus of Trichoderma viride was purchased from the Laboratory of Microbiology, University of Brawijaya, Indonesia while CM was obtained locally. The CM was ground finely to 1-2 mm particle size before used as a substrate. A fermentation procedure was applied in this study. After autoclave for 20 minutes at 20 psi, the CM was then cooled to room temperature before the inoculation of Trichoderma viride with 53.1 CFU/g/kg CM. The CM and T. viride were thoroughly mixed and added with distilled water to maintain 80% moisture content of the substrate. The autoclaved mixture was placed on the plastic tray with 2 cm thick and incubated for ten days. The fermented CM was harvested and then oven-dried for 24 hours at 60°C. The fermented CM was extracted to produce crude enzyme [13]. Trichoderma viride fermented CM was diluted with distilled water. The CM to water ratio was 1 kg CM to 5 litre water. The mixture was rotary-shaked for 1 h with a speed of 200 rpm. After rotary-shaking, the samples were filtered with muslin and the supernatant was then centrifuged with a speed of 2,500 rpm for 15 minutes. The residue was discarded and the fluid was obtained as a crude enzyme.

2.2. Birds and feeds
The study was carried out in the animal house at The Tadulako University, Palu, Indonesia. A total of 200 day-old male broilers were used and kept for six weeks. The chicks were placed in brooder pens for 14 days and transferred into the 25 cages from day 15. The chicks were fed starter diets from days 1 to 21 and grower diets from 21 to 42 days.

Diets were formulated by using the UFFC computer program version 1.11 [14]. The five experimental diets are described in table 1. A total of 50 ml crude enzyme was sprayed onto 10 kg feed to meet a 0.5% crude enzyme by using a sprayer when the diet was mixed in a horizontal feed mixer. The detailed treatment diets are shown in table 2.
On day 35, two birds from each replicate were placed in the metabolism cages for digestibility study. The birds were kept in the metabolism cages for one week. Excreta was collected daily on plastic trays placed under the wire battery floor after discarding any foreign material for three consecutive days (days 40 to 42). The excreta were oven-dried on the same day of collection. Representative samples of feed and excreta were collected to determine nutrient compositions.

2.4. Weights of digestive organs and chemical content of breast muscle
On day 42, two broilers from each replicate cage were randomly taken and individually weighed. The broiler chickens were slaughtered by cervical dislocation. Gizzard, liver, and heart were taken after being cleaned from fat. The empty weight of gizzard, liver, and heart were individually determined and expressed as g/100 g body weight. The breast meats from each replication were taken by dissection from the carcass. The meat samples were collected for chemical analysis.
2.5. Chemical analysis
The method of AOAC was used to analyze gross energy, crude protein, lipid and crude fiber [15]. The gross energy of the diets and excreta were analyzed using an adiabatic Bomb Calorimeter. Analysis of crude protein was done using the Micro Kjeldahl method. The crude fiber was measured by extracting with acid and alkali. Cholesterol content of breast meat was analyzed by using the Lieberman-Burchard reaction [15]. Acid insoluble ash (AIA) was used as a marker and analyzed [16]. The digestibilities of the protein and other nutrients were calculated by the formula:

\[
\% \text{ Apparent digestibility of nutrients} = \frac{(\text{Nutrients/AIA})_{\text{diet}} - (\text{Nutrients/AIA})_{\text{feces}}}{(\text{Nutrients/AIA})_{\text{diet}}} \times 100
\]

2.6. Statistical analysis
The study was designed using a completely randomized design with five treatment diets and five replications. Data were analyzed by variance analysis using the Minitab program [17]. The significance of difference found significant by variance analysis was separated by Tukey test [18].

3. Results and discussion
Data of digestibilities of protein, crude fiber, and metabolizable energy, the weights of liver, gizzard, and heart, the contents of protein, fat and cholesterol of breast meat of broiler chickens are in tables 3 and 4. The supplementation of crude enzymes from Trichoderma viride in the increasing levels of copra meal in the diet produced better digestibilities of protein, crude fibre, metabolizable energy of the diets and cholesterol content of the breast meat (table 5). However, the weights of the liver (P=0.302), gizzard (P=0.705), heart (P=0.241), abdominal fat (P=0.890), the percentage of protein (P=0.984) and lipid (P=0.364) of breast meat were not affected by treatments.

| Treatment | Protein (%) | Crude fiber (%) | AME (kcal/kg) |
|-----------|-------------|----------------|--------------|
| T-1       | 66.8<sup>a</sup> | 17.2<sup>a</sup> | 2854<sup>a</sup> |
| T-2       | 71.6<sup>b</sup> | 23.9<sup>b</sup> | 3008<sup>bc</sup> |
| T-3       | 74.6<sup>c</sup> | 27.6<sup>c</sup> | 3100<sup>c</sup> |
| T-4       | 75.7<sup>c</sup> | 30.8<sup>c</sup> | 3020<sup>c</sup> |
| T-5       | 70.8<sup>b</sup> | 24.3<sup>b</sup> | 2975<sup>b</sup> |
| SEM       | 0.420       | 0.797          | 21.9         |
| p-value   | >0.001      | >0.001         | >0.001       |

AME: Apparent metabolizable energy

Early findings indicated that the use of commercial enzymes in CM based diets increased the feed digestibilities [7]. In the current study, protein digestibility of the control diet without crude enzyme supplementation was about 67% (table 3). Protein digestibility increased as a result of crude enzyme supplementation, although the diets were added with increasing levels of copra meal. This possibly indicates that there was a greater hydrolysis of copra meal protein when the concentration of copra meal ranged between 10 and 15% in the diet. Above that level, the substrate of protein in the diet might be too much for the 0.5% crude enzyme concentration in the CM containing diets and thus reduced the protein digestibility. The relationship between the diets containing increasing levels of
CM supplemented with crude enzymes from *Trichoderma viride*, and protein digestibility followed the pattern of a curvilinear relationship with $Y = -1.514X^2 + 10.30X + 57.64$ and $r^2 = 0.894$ (figure 1). This finding was in accordance with the previous finding [19].

**Table 4.** Response of digestive organs of broilers fed the experimental diets (g/100 g body weight).

| Treatments | Parameters |
|------------|------------|
|            | Liver      | Gizzard    | Hearts     | Abdominal fat |
| T-1        | 1.76       | 1.70       | 0.37       | 1.23 |
| T-2        | 1.85       | 1.69       | 0.39       | 1.26 |
| T-3        | 1.83       | 1.71       | 0.39       | 1.26 |
| T-4        | 1.83       | 1.70       | 0.39       | 1.27 |
| T-5        | 1.84       | 1.83       | 0.40       | 1.28 |
| SEM        | 0.34       | 0.0344     | 0.008      | 1.26 |
| p-value    | 0.302      | 0.705      | 0.241      | 0.890 |

**Table 5.** Protein, fat, and cholesterol contents of breast meat of birds fed the experimental diets.

| Treatments | Parameters |
|------------|------------|
|            | Protein (%) | Lipid (%) | Cholesterol (mg/100g) |
| T-1        | 23.26       | 0.76      | 0.72$^a$ |
| T-2        | 23.53       | 0.73      | 0.48$^c$ |
| T-3        | 23.29       | 0.65      | 0.51$^c$ |
| T-4        | 23.29       | 0.66      | 0.52$^c$ |
| T-5        | 23.28       | 0.68      | 0.54$^b$ |
| SEM        | 0.146       | 0.020     | 0.022      |
| p-value    | 0.984       | 0.364     | >0.001     |

**Figure 1.** Effect of the experimental diets supplemented with crude enzymes on protein digestibility.

The same pattern on the relationship between the experimental diets and digestibility of crude fiber was found in this study ($Y=1.924X^2 + 13.64X + 5.006$ and $r^2= 0.831$; figure 2). Crude fiber
digestibility curvilinearly increased from 17.2% in the control diet to 30.8% in a 15% copra meal diet. Further increase in the inclusion of copra meal to 20% in the diet decreased crude fiber digestibility to 24.3%. Breaking down the fiber backbone through crude enzyme supplementation may have been the reason why the digestibility of crude fiber increased. The crude enzyme produced and used in the current study contains fiber degrading enzymes, particularly cellulases, being between 0.79 and 0.89 g glucose/l [20]. Fiber fractions found in the copra meal were mainly in the form of mannan and cellulose [21]. Accordingly, when copra meal was used as a substrate of fermentation, these two fractions need to be hydrolyzed by the enzymes of cellulase and mannanase produced by microorganisms, and thus, these two enzymes might present in the *Trichoderma viride* – fermented copra meal.

The improved apparent metabolizable energy due to increasing levels of copra meal and crude enzyme supplementation found in this study may be partly due to the increase in the digestibility of two fractions of nutrients, protein and crude fiber. The data indicated that the efficacy of this crude enzyme to digest crude fiber could not be maintained in the 20% copra meal diet and this finding was supported by the previous finding [22], indicating that when the enzyme concentration is kept constant and the substrate concentration is increased gradually, the reaction velocity will reach the maximum level. In the current study, the maximum hydrolysis may occur when the level of CM was 15% in the diet and increased copra meal level could deteriorate the digestibility of the crude fiber.

The effects of commercial enzymes on the relative weights of gizzard of birds fed different diets have been reported [23]. The previous study indicated that The supplementation of CM diet with commercial enzymes produced a smaller relative gizzard weight in broiler chickens due to the fact that enzymes decreased the gizzard load in grinding the feed [23]. However, the current study shows that the inclusion of crude enzyme in this particular diet could not decrease the relative weight of gizzard. The relative weights of gizzard were between 1.70 and 1.83 g/100 g body weight and the range of these weight of gizzard was not statistically different. The weights of heart and liver were not also affected by the experimental diet.

It has been long believed that meat with a lower fat and cholesterol contents and higher in protein content as desired by consumers. The effects of diets on protein and lipid contents in the breast meat were not statistically detected in the current study. Protein contents of breast meat of broiler in the present study were about 23% and the fat contents ranged between 0.65% and 0.76%. Cholesterol content, on the other hand, was decreased due to either crude enzyme supplementation or copra meal.

![Figure 2](image_url)  
**Figure 2.** Effect of experimental diets on crude fibre digestibility.
addition. The reduction in cholesterol content in the diet containing copra meal and crude enzyme supplementation is hard to be explained due to the difficulty of determining whether the reduction of cholesterol content in the breast meat was a result of copra meal or crude enzyme. Further study is needed to clarify this finding.

4. Conclusions
1. The supplementation of increasing levels of CM in the diets with a crude enzyme produced better nutrient digestibility and lower cholesterol content of the breast meat than the 0% copra meal diet without enzyme supplementation.
2. The response of the protein and crude fiber digestibilities were curvilinearly over the increasing levels of CM in the rations and enzyme supplementation.

5. Acknowledgments
We wish to express special gratitude to a Staff at Poultry Unit, The University of Tadulako, Mr Anto, who diligently takes care of the birds and routinely clean up the pens. We would also like to thank the DIKTI (Indonesian Higher Education Directorate) for the financial aid for doing this experiment and publishing this article.

References
[1] Sundu B, Hatta U and Chaudhry A S 2012 Potential use of beta-mannan from copra meal as a feed additive for broilers Worlds Poult. Sci. J. 68 707–16
[2] Thomas O A and Scott M L 1962 Coconut oil meal as a protein supplement in practical poultry diets Poult. Sci. 41 477–85
[3] Panigrahi S, Machin D H, Parr W H and Bainton J 1987 Responses of broiler chicks to dietary copra cake of high lipid content Br. Poult. Sci. 28 589–600
[4] Sundu B, Kumar A and Dingle J 2005 Comparison of feeding values of palm kernel meal and copra meal for broilers Recent Adv. Anim. Nutr. Aust. 15 16A
[5] Sundu B 2009 Gastro-intestinal response and passage time of pelleted diets in digestive tract of broilers Int. J. Poult. Sci. 8 976–9
[6] Eustace A, Iyayi and Davies B I 2005 Effect of enzyme supplementation of palm kernel meal and brewer’s dried grain on the performance of broilers Int. J. Poult. Sci. 4 76–80
[7] Sundu B, Kumar A and Dingle J 2006 Response of broiler chicks fed increasing levels of copra meal and enzymes Int. J. Poult. Sci 5 13–8
[8] Hernandez N, Rodriguez-Alegria M E, Gonzalez F and Lopez-Munguia A 2000 Enzymatic treatment of rice bran to improve processing J. Am. Oil Chem. Soc. 77 177–80
[9] Pluske J R, Moughan P J, Thomas D V, Kumar A and Dingle J G 1997 Releasing energy from rice bran, copra meal and canola in diets using exogenous enzymes Proceedings of the 13th Annual Alltech symposium (Nottingham University Press Nottingham, UK) pp 81–94
[10] Filler K 2001 Production of enzymes for the feed industry using solid substrate fermentation Proceedings of Alltech’s 17th annual symposium ed T P Lyons and K A Jacques (Nottingham, UK: Nottingham University Press) pp 131–54
[11] Kubicek C P 1992 The cellulase proteins of Trichoderma reesei: structure, multiplicity, mode of action and regulation of formation Enzymes and Products from Bacteria Fungi and Plant Cells (Springer) pp 1–27
[12] Ikram-ul-Haq, Javed M M, Khan S and Siddiq T Z 2005 Cotton saccharifying activity of cellulases produced by co-culture of Aspergillus niger and Trichoderma viridae Res. J. Agric Biol. Sci 1 241–5
[13] Jacob N and Prema P 2006 Influence of mode of fermentation on production of polygalacturonase by a novel strain of streptomyces lydicus Food Technol. Biotechnol. 44 263–7
[14] Pesti G M, Miller B R and Chambers R 1986 User Friendly feed Formulation Program (UFFF) version 1.11-256 k Dep. Poult. Sci. Agric. Econ. Univ. Georg. Atlanta
[15] AOAC 2000 *Association of Official Analytical Chemist Official Methods of Analyses* (Washington, DC: AOAC International)

[16] Siriwan P, Bryden W L, Mollah Y and Annison E F 1993 Measurement of endogenous amino acid losses in poultry *Br. Poult. Sci.* **34** 939–49

[17] Minitab I N C 2003 MINITAB User’s guide 2: data analysis and quality tools 2003

[18] Steel R and Torrie J 1991 *Prinsip dan Prosedur Statistika* Penerjemah B. Sumantri (Jakarta: PT. Gramedia Utama)

[19] Saropah D A, Jannah A and Maunatin A 2013 Kinetika reaksi enzimatis ekstrak kasar enzim selulase bakteri selulolitik hasil isolasi dari bekatul *Alchemy* **2** 34-45

[20] Hatta U, Sjofjan O, Subagiyo I and Sundu B 2014 Effects of fermentation by Trichoderma viride on nutritive value of copra meal, cellulase activity and performance of broiler chickens *Livest. Res. Dev* **26**

[21] Balasubramaniam K 1976 Polysaccharides of the kernel of maturing and matured coconuts *J. Food Sci.* **41** 1370–3

[22] Alam M Z, Manchur M A and Anwar M N 2004 Isolation, purification, characterization of cellulolytic enzymes produced by the isolate Streptomyces omiyaensis *Pakistan J. Biol. Sci.* **7** 1647–53

[23] Sundu B, Kumar A and Dingle J 2008 The effect of proportion of crumbled copra meal and enzyme supplementation on broiler growth and gastrointestinal development *Int. J. Poult. Sci.* **7** 511–5