

The Use of “Organic Protein” in Feed Formulation on Intestinal and Ammonia Levels of Broiler

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Abstract. The aim of this study is to determine the effect of Organic Protein as a feed ingredient on pH, viscosity and enzyme activity in the small intestine and levels of ammonia in broiler excreta. Organic Protein is a Single Cell Protein (SCP) product name from PT. Miwon Indonesia that has a protein content up to 40%. We conducted a field experiment and used DOC broilers and Organic Protein as treatment feed in a Completely Randomized Design (CRD) comprising of 5 treatments and 6 replicates, accounting for 30 experimental units. The feed treatments were basal feed T0, T1, T2, T3 and T4 added with Organic Protein 0%, 1.5%, 3%, 4.5%, and 6%, respectively. The observed variables were pH, viscosity, enzyme activity (amylase and protease) of small intestine and excreta ammonia level of broiler. The data were analyzed with ANOVA followed by Duncan's Multiple Range Test (DMRT). The results showed the treatments have very significantly different effects ($P < 0.01$) on the amylase and protease activities, but significantly different effects ($P > 0.05$) on pH, viscosity of small intestine, and ammonia levels of excreta. The use of Organic Protein at a level of 4.5% gave the best results to increase enzyme activity of amylase and protease.

Keywords: Organic protein, pH, viscosity, enzyme activity, ammonia content, broiler

Abstrak. Penelitian ini bertujuan untuk mengetahui pengaruh Organik Protein sebagai bahan pakan terhadap pH, viskositas dan Aktivitas enzim pada usus halus serta kadar ammonia ekskreta ayam pedaging. Organic Protein adalah sebuah nama produk Protein Sel Tunggal (PST) dari PT. Miwon Indonesia yang memiliki kandungan protein hingga 40%. Materi penelitian yang digunakan adalah 480 ekor DOC ayam pedaging dan “Organic Protein” sebagai pakan perlakuan. Penelitian ini dilakukan dengan menggunakan metode percobaan lapang dengan Rancangan Acak Lengkap (RAL). Penelitian dilaksanakan dengan 5 perlakuan dan 6 ulangan sehingga terdapat 30-unit percobaan. Variabel yang diamati adalah pH, viskositas, enzim amilase dan protease usus halus, serta kadar amonia ekskreta ayam pedaging. Pakan perlakuan yang digunakan adalah: pakan basal, dan ditambahkan Organic Protein secara berurutan yaitu: T0, T1, T2, T3, dan T4 sebanyak 0%; 1,5%; 3%; 4,5%; dan 6%. Analisis statistik yang digunakan adalah ANOVA, dilanjutkan dengan Uji Jarak Berganda Duncan's (UJBD). Hasil penelitian menunjukkan bahwa perlakuan mampu memberikan pengaruh berbeda sangat nyata ($P < 0.01$) terhadap aktivitas enzim amilase dan protease, tetapi memberikan pengaruh berbeda tidak nyata ($P > 0.05$) terhadap pH, viskositas usunnj halus, dan amonia ekskreta ayam pedaing. Penggunaan Organic Portein pada kadar 4,5% memberikan hasil terbaik untuk meningkatkan aktivitas enzim amilase dan protease.

Kata Kunci: Organic Protein, pH, viskositas, aktivitas enzim, kadar amonia, ayam pedaging

Introduction

For years, the consumption of broiler meat consumption has exceeded that of beef meat; per capita per year consumption of broiler and beef in 2019 reached 12.79 kg/year and 2.66 kg/year, respectively, indicating an increase from the 2018's 5.55kg and 2.56, respectively (BPS, 2020). Feed is the main determining factor of the success of broiler rearing, which takes up 60-80% of the total production cost. Considering the very expensive cost for high-protein feeds, it

takes efficient utilization of feed in order to prevent negative interference with productivity.

Organic Protein is a product brand of PT. Miwon Indonesia, processed from by-products of MSG which has been fermented with *Brevibacterium* known as the Single Cell Protein. Single Cell Protein (SCP) is a product of single organism cell biomass with a relatively high content of protein, amino acids, vitamin B complex (Sjojfan et al., 2020). Compared to fish meal, most SCP sources have similar level of

lysine, methionine and cystine, as well as higher proportions of tryptophan and threonine. Single-cell proteins or microbial proteins refer to edible unicellular microorganisms. The biomass or protein extract from pure or mixed cultures of algae, yeasts, fungi or bacteria may be used as an ingredient or a substitute for protein-rich foods and is suitable for either human consumption or animal feeds (Adli et al., 2020).

Samadi et al (2012) reported that while 6% substitution of PST CJ Prosin did not affect body weight gain, feed consumption ratio (FCR) of chickens, the 12% and 18% level have reduced body weight and produced high feed conversion values. Chand et al (2014) stated that maximum body weight was recorded in the yeast SCP group at a level of 10.5 grams/kg feed which positively improved broiler performance. The gap in these results is due to the use of different microorganisms. In this study, we investigate the effect of Organic Protein in feed on pH and viscosity, the enzyme activity of small intestine digesta, and ammonia levels in broiler excreta.

Materials and Methods

Research Material

We used 480 DOC broilers, unsex, and reared from the age of 1 to 35 days. The feed used was commercial basal feed BR1 and BR2 added with Organic Protein manufactured by PT. Miwon Indonesia. Data of the nutrient content of the treatment feed are presented in Table 1. The DOC were kept in 30-unit litter cages, each measuring 150 x 120 x 70 cm and containing 16

broilers. We sampled the small intestine digestion from the jejunum to the ileum for measuring pH, viscosity, and enzyme activity. We sampled excreta to measure ammonia level.

Research methods

We applied field experiment in a Completely Randomized Design (CRD) and analyzed the data using ANOVA with 5 treatments and 6 replications to make up 30 experimental units. The treatment feed was given from DOC on the first day until the 35th day. The feed treatments were:

- T0 = 100 %basal feed
- T1 = 98.5 % basal feed + 1.5% Organic Protein
- T2 = 97% basal feed + 3% Organic Protein
- T3 = 95.5 % basal feed + 4.5% Organic Protein
- T4 = 94% basal feed + 6% Organic Protein

Observed Variables

The variables observed in this study included:

- a. pH of small intestine digesta (pH Meter)
- b. Viscosity of small intestine digesta (CPs) – Digital Rotary Viscometer (Brookfield)
- c. Amylase enzyme activity of small intestine ($\mu\text{mol} / \text{g} \text{ minute}$) – Nelson Somogyi (1952)
- d. Protease enzyme activity of small intestine ($\mu\text{mol} / \text{g} \text{ minute}$) – Bergemeyer and Grassl (1985)
- e. Excreta ammonia level (ppm) – Microdiffusion Conway

Table 1. Nutrient content of treated feed

Content	Starter ¹⁾					Finisher ²⁾				
	T0	T1	T2	T3	T4	T0	T1	T2	T3	T4
Dry matter (%)	89.89	89.33	88.43	86.10	89.20	90.45	89.20	88.98	88.61	87.65
Crude protein (%)	22.44	23.71	23.04	23.43	23.40	18.10	18.71	18.90	19.43	20.43
Fat (%)	6.37	6.02	5.41	5.47	5.89	-	-	-	-	-
Crude fiber (%)	8.07	4.83	5.13	4.59	4.60	-	-	-	-	-
Ash (%)	4.98	5.15	5.35	5.35	5.53	-	-	-	-	-
ME (kcal/kg)	2914.8	2889.6	2828	2816.1	277.6	-	-	-	-	-

Source:

1) Feed Laboratory of Department of Animal Husbandry and Fisheries, Blitar District (2021)

2) Laboratory of Nutrition and Animal Feed, Faculty of Animal Science, Universitas Brawijaya

Data analysis

Data were recorded and calculated using the program *Microsoft Excel*, and then subjected to ANOVA in a Completely Randomized Design (CRD). Given a significantly different result ($P < 0,05$) or very significant ($P < 0,01$), we continued with Duncan's Multiple Distance Test (DMRT).

Results and Discussion

The results of supplementing Organic Protein on feed and its effect on pH, viscosity, and enzyme activity (amylase and protease) of the small intestine and levels of ammonia excreta in broiler are presented in Table 2.

Effect of Treatment on pH of the Small Intestinal Digesta

The results of the analysis of variance showed that Organic Protein had no significant effect ($P > 0.05$) on pH of broilers' ileal digesta. We observed the lowest pH value in T4 (6.68 ± 0.33) and the highest in T3 (6.92 ± 0.29). At 6%, Organic Protein was able to lower the pH of the small intestine.

The intestinal pH level is a factor that makes up a particular microbial population and also affects the digestibility and absorption value of most nutrients. Ripon et al., (2019) stated that most pathogens grow at pH close to 7 or slightly higher. Decreasing pH value of the digestive tract is known to reduce pathogenic bacteria, such as

Escherichia coli and *Salmonella*. Acidic conditions of the small intestine will reduce the growth of pathogenic bacteria, thus improving the condition of the digestive tract and nutrient digestibility, which eventually improve feed rate in the small intestine in the process of nutrient absorption (Puspasari et al., 2016).

The similarity of the parts of the digestive tract affects the life of digestive microbes which are closely related to the products of digestive enzymes and enzymes from microorganism contained in feed (Sjojfan et al, 2020). Gastric acid (HCl) will be released by digestive juice naturally to form acidic conditions, and stomach acid conditions serve as a selection of microbes that will enter the intestine.

Effect of Treatment on Viscosity of Small Intestinal Digesta

Sjojfan et al, (2015) revealed that viscosity is the resistance to flow from a system caused by shear. The results of analysis of variance showed that Organic Protein had no significant different effect ($P > 0.05$) on viscosity of the small intestine of broilers. Table 2 showed that Organic Protein did not affect the viscosity value of intestinal digesta, which may be due to the low viscosity of the Organic Protein. High viscosity can overload the intestines to absorb feed nutrients. The increase of viscosity value of the small intestine can reduce the efficiency of digestion.

Table 2. Average value of treatment on pH, viscosity, and activity of intestinal enzymes and ammonia levels of broilers excreta

Research Variable	Treatment				
	T0	T1	T2	T3	T4
pH of Small Intestinal Digesta	6.78±0.50	6.87±0.37	6.90±0.49	6.92±0.29	6.68±0.33
Viscosity of Small Intestinal Digesta (CPs)	3.33±0.82	3.17±0.75	3.17±0.41	3.08±0.66	3.50±0.55
Enzyme Amylase (µmol /g minute)	549.97±25.02 ^{BC}	502.19±11.86 ^C	670.825±47.64 ^A	581.67±91.03 ^B	547.81±50.08 ^C
Enzyme Protease (µmol /g minute)	5.21 ± 0.02 ^C	4.99 ± 0.54 ^{CD}	3.95 ± 0.40 ^D	6.62 ± 1,18 ^B	7.89 ± 0.08 ^A
Ammonia Level (ppm)	0.118 ± 0.13	0.233 ± 0.11	0.238 ± 0.24	0.34 ± 0.15	0.40 ± 0.14

Note: Values bearing different superscript (A-D) within row show a significantly different effect ($P < 0.01$)

Widodo (2010) emphasized that an increase in digesta viscosity will have an impact on the difficulty of the digesta to be digested, especially because of the difficulties experienced by enzymes to penetrate the surface or matrix of the digesta. Low viscosity indicates the nature of amino acids (the result of protein hydrolysis) which are soluble in water (Fitasari, 2011).

High viscosity indicates that chyme in the intestine is more viscous, and thus the rate of digestion runs slowly and causes under-optimum absorption. Based on the research of Olfati et al., (2021) the use of gelatin increases the viscosity of the ileal digesta which causes a decrease in the performance and digestibility of nutrients. The determining factors to viscosity are temperature, pressure, weight, solution molecules, concentration of solution and dissolved materials.

Effect of Treatment on Amylase Enzyme Activity of Small Intestine

The results of the analysis of variance showed that the use of Organic Protein in the feed had a highly significantly different effect ($P < 0.01$) on the activity of amylase enzyme in the small intestine. Table 2 shows the average value of the highest enzyme activity in T2 treatment was $670,825 \pm 47,64$ supplemented with 3% Organic Protein. The amylase enzyme activity decreased when 1.5% and 6% Organic Protein was used in the treatment compared to the control.

The decrease in amylase enzyme levels may be due to the presence of inhibitory factors in the Single Cell Protein (SCP). SCP is known to have a high protein content as well as high nucleic acid content. Nucleic acids are macromolecules that undergo different metabolism from protein and have low digestibility (Samadi, et al. 2012), but at a high level, nucleic acids in the SCP can cause toxicity to broilers due to its degradation through a long chain reaction and results in the formation of free radicals (Ludfi, 2012). Sugiantoningsih (2012) states that the work of enzyme activities

can be influenced by temperature, pH, concentration of enzymes, substrates, cofactors and enzyme inhibitors. At an optimum pH, the enzyme can decompose the substrate maximally.

Amylase is an enzyme that digests carbohydrates (polysaccharides) and starch into simpler food substances such as glucose (Lehninger, 1994; Nurhayatin, 2016). Carbohydrates are converted into simpler form and utilized as a source of energy for chicken.

Effect of Treatment on Protease Enzyme Activity of Small Intestine

The results of the analysis of variance showed that the use of Organic Protein in feed had a highly significant different effect ($P < 0.01$) on the activity of the protease enzyme in the small intestine. The average values of protease enzyme activity from the lowest to the highest were observed in T4 (7.89 ± 0.08), T3 (6.62 ± 1.18), T0 (5.21 ± 0.02), T1 (4.96 ± 0.54) and T2 (670.83 ± 0.40), respectively. The activity of protease enzymes increased at the level of 4.5% and 6% of Organic Protein. This increase in enzyme activity is in line with Sugiantoningsih (2012) that single-cell protein fermented with *brevibacterium* can help increase protease activity in the digestive tract during food absorption in the small intestine because it contains high levels of protein and free amino acid compounds. The value of enzyme activity can be influenced by pH, concentration, temperature, and substrate. Enzyme activity works optimally at neutral pH.

Protease degrades protein into amino acids in the body. Amino acids are absorbed by the chicken and then converted into body protein. It is in accordance with Fitasari (2011) amino acids are indispensable for broilers in the formation of meat. Organic Protein has high protein, amino acids and vitamins which can be used as animal feed to improve amino acid deficiency. SCP has good quality and protein digestibility, and thus

feasible at certain level to substitute fish meal in feed (Ludfi, 2012).

Effect of Treatment on Levels of Ammonia Excreta

The results of the analysis of variance showed that the use of Organic Protein had no significantly different effect ($P>0.05$) on ammonia level of broiler excreta. The complete data on the effect of treatment on the excreta ammonia levels (Table 6) showed that the level of ammonia increase with the percentage of Organic Protein. However, this increase remained in the normal range of NH_3 levels in broilers, which was below 20 ppm.

Measurement of ammonia levels indicates whether the feed protein can be completely digested by livestock. The formation of ammonia in livestock body occurs in the swab as the main site for hydrolyzing urea into ammonia. The efficiency food absorption, especially proteins and amino acids, can be attributed to the production of NH_3 (Angkeke et al., 2019). Protein that is not absorbed in the digestive tract will be converted into uric acid and then excreted with feces (Hutauruk, 2017).

Ammonia excreta is an alkaline gas produced by livestock which is colorless, toxic and highly irritating, thus generating air pollution in livestock farm (Soma, 2016). The source of air pollution in broiler farms comes from the manure that is related to the nitrogen and sulfide elements contained in the manure, which at the time of accumulation of manure or storage there is a decomposition process by microorganisms to form high content of ammonia gas and hydrogen sulfide (H_2S) gas, dimethyl sulfide, carbon disulfide, and mercaptans which simultaneously produce odor. It is because hydrogen sulfide (H_2S) is a gas that can produce an unpleasant odor (Nugrahani et al, 2016). At a level of 25 ppm, ammonia interferes with chicken breathing. It will get worse, if it is in a poor ventilation housing (Wihandoyo et al., 2001).

Conclusions

The use of Organic Protein in feed can increase the activity of amylase and protease but does not decrease pH and viscosity of the small intestine as well as the excreta ammonia level of broiler.

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