THE EFFECT OF "ORGANIK PROTEIN" IN FEED ON pH, VISCOSITY, AND ENZYMES ACTIVITY OF INTESTINAL AND EXCRETA AMMONIA LEVELS OF BROILER

UNDERGRADUATE THESIS

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UNDERGRADUATE ANIMAL SCIENCE PROGRAM
FACULTY OF ANIMAL SCIENCE
UNIVERSITAS BRAWIJAYA
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Undergraduate thesis as one of the requirements for a
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Universitas Brawijaya

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The Author

THE EFFECT OF "ORGANIK PROTEIN" IN FEED ON pH, VISCOSITY, AND ENZYMES ACTIVITY OF INTESTINE AND EXCRETA AMMONIA LEVELS OF BROILER

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ABSTRACT

The aim of this study was to determine the effect of Organik protein in feed on pH, viscosity, and enzymes activity (amylase and protease) in the small intestine digest and ammonia excreta level of broiler. Organik protein is a feed ingredient that has a single-cell protein (SCP) component. Single-cell protein has a high content of protein, amino acids, and B-complex vitamins. The research material used was 480 broilers DOC and Organik protein as treatment feed. This research was conducted using a field experiment method in Completely Randomized Design (CRD). The research was carry-out with 5 treatments and 6 replications so that there were 30 experimental units. The variables observed were pH, viscosity, amylase and protease enzyme of intestine, and ammonia excreta level of broiler. The treatment feed used was: (p0) basal feed, and the Organik protein added

sequentially, namely: P1, P2, P3, and P4 (1,5%; 3%; 4,5%; and 6%). Statistical analysis used was ANOVA, followed by Duncan's Multiple Range Test (DMRT) if the result showed significantly different (P<0,05) or highly significant different (P<0,01). The results showed that the treatment was able to have a highly significant different effect (P<0.01) on the enzymes activity amylase and protease, but had no significant different effect (P>0.05) on pH, viscosity of small intestine digest, and ammonia levels of excreta. The use of Organik protein at a level of 4.5% gave the best results to increase enzyme activity of amylase and protease, as well as decrease on viscosity of small intestine digesta.

Keywords: Organik protein, pH, viscosity, enzyme activity, ammonia content, broiler.

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SUMMARY

Broiler meat is the most consumed source of animal protein in Indonesia. Consumption of broiler meat is much higher than beef consumption from year to year. The high demand for animal protein in Indonesia requires good broiler maintenance to produce good broiler meat products. Feed takes up 60 – 80% of the total production cost. Protein is one of the important feed ingredients in growth and determining the efficiency of feed use, but it has a high price. The high cost of feed requires farmers to use feed efficiently to get good production at a low price. Organik protein is a product with a protein content of up to 40%, and can be obtained at a low price. Single cell protein, one of the components in Organik protein, is a single organism cell biomass which is rich in amino acids and vitamin B complex. Organik protein is obtained from the fermentation of MSG by-products with bevibacteium bacteria so that it has value and can be used as a

protein source in animal feed. Single cell protein has the same lysine, methionine and systems compared to fish meal and high in tryptophan and threonine.

The purpose of this study was to determine the effect of the use of Organik protein in feed on the pH, viscosity and activity of enzymes (amylase and protease) in the small intestine digesta and levels of ammonia in broiler excreta. The material used were 480 DOC broilers, undifferentiated by sex, reared from the age of day-1 to 35 days. The treatment feed used was Organik protein by-product of MSG processing by PT. Miwon Indonesia which has a protein content of up to 40%. The basal feed used was commercial feed BR1 and BR2 produced by PT. Japfa Comfeed TBK. The treatment feed used was: (p0) basal feed, and the Organik protein added sequentially, namely: P1, P2, P3, and P4 (1,5%; 3%; 4,5%; and 6%). The data were analyzed using ANOVA from a Completely Randomized Design (CRD). If the results are significantly different (P<0,05) or highly significant different (P<0,01), then it is continued with Duncan's Multiple Distance Test (DMRT).

The results of the analysis of variance had no significantly different effect (P>0,05) on pH, viscosity of small intestine digesta, and excreta ammonia levels and had highly significant different effect (P<0,01) on amylase and protease enzyme activity of small intestine broiler. the use of 3% (670.825±47.64) and 4.5% (581.67±91.03) OP in the feed had a significant effect and increased the activity of the intestinal amylase enzyme. while the protease activity increased and had a significant effect on the use of OP as much as 4.5% (6.62 ± 1.18) and 6% (7.89 ± 0.08). Statistically OP did not have an effect on viscosity, but in terms of the use

of OP at the level of 4.5% it was able to reduce the viscosity of intestinal digesta compared to control feed. Excreta ammonia content increases with increasing use of OP in feed

It can be concluded that the use of Organik protein in feed up to a level of 4.5% was able to increase the activity of enzymes of amylase and protease, but did not decrease the pH and viscosity of small intestine as well as excreta ammonia levels. Based on this research, it is recommended to use Organik protein as a protein source for broiler at a level of 4.5%.

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LIST OF ABBREVIATIONS AND SYMBOLS

%	: percentage
BPS	: Badan Pusat Statistik
cm	: centimeter
CRD	: Complete Randomized Design
DOC	: *Day Old Chick*
Etc.	: et cetera
Et al.	: et alii
FCR	: Feed Conversion *Ratio*
g	: gram
H2S	: Hidrogen Sulfide
Kg	: Kilogram
Kcal/kg	: Kilocalori/ kilogram
Max	: Maximum
ME	: Metabolic Energy
Min	: Minimum
MSG	: Monosodium Glutamate
NH3	: Ammonia/ Hidrogen nitrida
NH4+	: Ammonium
OP	: Organik protein
pH	: potential of Hydrogen
RPM	: Revolutions per minute
SCP	: Single Cell Protein
SNI	: Standar Nasional Indonesia
oC	: Celsius Degrees
µg	: microgram
µm	: micrometer
µl	: microliter
µmol	: micromole

CHAPTER I
INTRODUCTION

1.1. Background

Animal protein food has an important role in fulfilling good nutritional needs. The most accessible supplier of animal protein for the community is broiler meat. Consumption of broiler meat in 2019 was 0,124 kg per capita a week, much more than beef consumption of 0,009 kg per capita per week (BPS, 2020). The high demand for animal protein in Indonesia requires good broiler maintenance to produce good chicken meat products. Broiler production can be considered in several aspects, including production performance, feed nutrition, and health status. The quality of broiler production will be good if it is maintained properly, such as environmental factors, high-quality feed, a good cage system, as well as health maintenance and disease prevention. The benchmark for the success of a livestock business is influenced by several factors such as the selection of superior breed, quality feed, and housing management.

Feed is the main factor in determining broiler rearing. Feed takes up 60 – 80% of the total production cost. Protein is one of the important elements in feed that is needed for the growth and efficient use of poultry feed. Feed ingredients that are high in protein are pricey. So that the use of feed ingredients must be efficient so as not to interfere with productivity. One of the efforts to produce poultry feed at an affordable price is to use additional by-products from processed agricultural materials to meet the nutritional needs of livestock. Natsir et al. (2010) state that efforts to reduce the

highest cost in the livestock business need to utilize local materials and wastes, both by-products of processing products, agricultural waste, livestock, and industrial waste.

The addition of feed additives is done to improve the appearance of poultry production, including drugs, antibiotics, or growth hormones. The addition of feed additives in animal feed has long been done to stimulate growth and prevent disease. The provision of feed additives can also be used as a substitute for antibiotics to increase the productivity and efficiency of the feed used. Synthetic amino acids used as additional feed ingredients are expected to increase the efficiency of feed intake.

Organik protein in feed is expected to meet the nutritional needs of broiler feed at affordable prices. Organik protein in the feed was used as a protein source feed ingredient. One indication of good feed digestion in the poultry digestive tract is observing intestinal characteristics i.e., viscosity and enzyme activity, as well as internal organ weight (Nurliana et al., 2019). In this study, the effect of the use of Organik protein in feed on pH, viscosity, and enzyme activity of the small intestine and excreta ammonia levels in broiler will be determined.

1.2. Problems

Based on the description of the background above, the research problem is how the effect of the use of Organik protein in feed on pH, viscosity, enzyme activity including amylase and protease small intestine and levels of ammonia excreta in broilers?

1.3. Purpose

To determine the effect of the use of Organik protein in feed on pH, viscosity, and enzymes activity (amylase and protease) of small intestine digesta and excreta ammonia levels of broiler.

1.4. Advantages

This research is expected to be useful as information for the community and breeders about the effect of using Organik protein in feed on pH, viscosity, and enzyme activity (amylase and protease) of the small intestine as well as levels of ammonia in broiler excreta and is expected to serve as the next groundwork of protein source feed ingredients.

1.5. Research Framework

One of the factors in determining the success of the livestock business is the efficient use of feed. Good feed will support the growth of goof livestock breeds. Protein is one of the main components of feed that contributes to the cost of poultry feed (Sari et al., 2014). Traditional protein sources are predicted to be increasingly scarce because they are used by humans too and are increasingly expensive. Farmers must use feed efficiently so that productivity is not compromised. One way to increase feed efficiency is to add feed additives to improve enzymatic digestion in the digestive tract (Primacitra et al., 2014). Organik protein is an ingredient that can be used as a protein source feed material at a low price.

Organik protein is a single-cell protein (SCP) known as a single-cell food microorganism in which the biomass or protein extract is derived from pure or mixed microscopic algae, yeast, fungal, and SCP bacteria. (Spalvins et al., 2018).

Yeast is one of the best microorganisms for single-cell protein production because of its nutritional quality and can coexist with animal protein (Adedayo et al., 2011; Chand et al., 2014). Yeast has a balanced proportion of amino acids, vitamin B complex, and also has probiotic properties making it more suitable for poultry feed (Chand et al., 2014).

Several studies on single-cell protein in livestock have been carried out but obtained a different result. The difference in results was caused by the use of different microorganisms. In the study of Samadi et al. (2012), the use of 6% SCP in commercial feed did not show a significant different effect but with 12% SCP there was a weight loss of about 600 grams. The use of SCP was tolerated in 6% used. In the study of Chand et al., 2014 the maximum bodyweight was recorded in the group receiving SCP yeast at a level of 10.5 grams/kg feed, and the use of SCP yeast improved broiler performance positively.

Single-cell protein is a cheaper source of dietary protein, as it can be produced from various substrates (sugar, molasses, cellulose, starch, etc.) on a large scale in the industry. The production and activity of enzymes are influenced by the intake and quality of nutrients, especially amino acids (protein). Compared with fish meal, most SCP had similar lysine, methionine, and cystine content and higher proportions of tryptophan and threonine. These amino acids are some of the essential amino acids needed by livestock. In this study, the use of Organik protein with high protein content and amino acids needed by broiler is expected to maximize chemical digestion so that the use of feed can be more efficient.

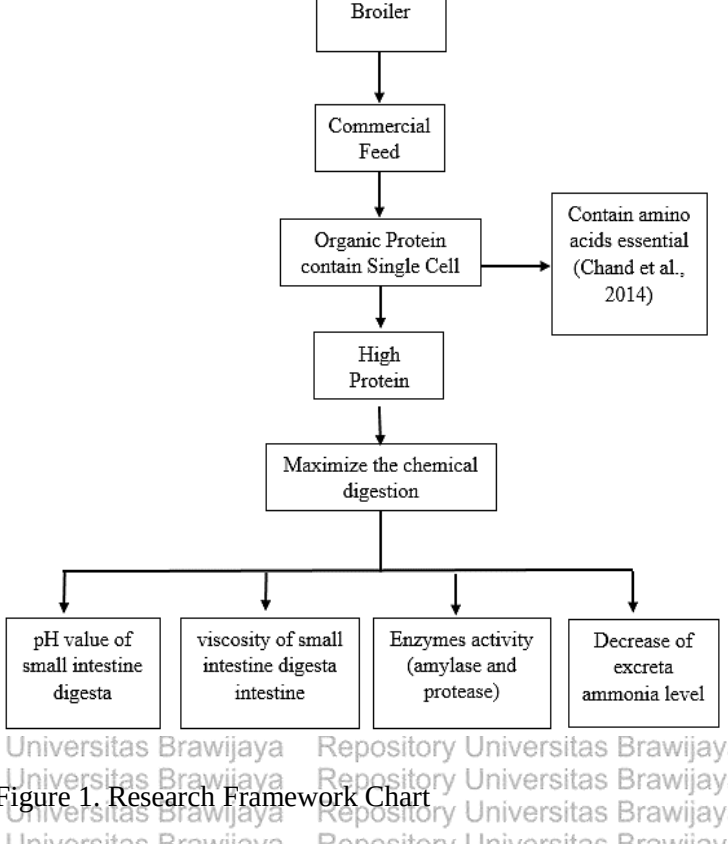


Figure 1. Research Framework Chart

1.6. Hypothesis

The use of Organik protein in feed can reduce on pH, viscosity of small intestine digest and level of excreta ammonia, as well as increase the activity of enzymes (amylase and protease) of the small intestine of broiler.

CHAPTER II
LITERATURE REVIEW

2.1. Single Cell Protein

Single Cell Protein (SCP) is one component of animal feed ingredients with a fairly high protein content and complete enough of amino acids, so it has the potential to be used as monogastric animal feed (Samadi et al., 2012). Sjofjan et al., (2020) confirmed that SCP is a single organism cell biomass product with relatively high content of protein, amino acids, and vitamin B complex. Goldberg (2013) revealed that microorganisms suitable for single cell protein production are divided into four main categories: bacteria, yeast, mold/fungi and algae. Bacteria also have a long history in the manufacture of SCP, especially for animal feed. Ritala et al., (2017) stated that SCP products derived from algae, fungi (including yeast) and bacteria are all being developed. The production stages generally include (a) preparation of nutrient media, possibly from waste, (b) cultivation, including solid fermentation, (c) separation and concentration of SCP, in some cases drying, and (d) final processing of SCP into material and products. SCP bacterial is still limited in the feed industry, if not including *cyanobacteria* products and non-photosynthetic bacteria. Some bacterial SCP is currently a by-product of other industries such as monosodium glutamate production, and this type of feed product is expected to increase with the expansion of biorefineries, as with yeast (Ritala et al., 2017).

SCP is frequently seen as a potential co-product that could strengthen the economic potential of an otherwise unprofitable biorefinery process, as well as a means of

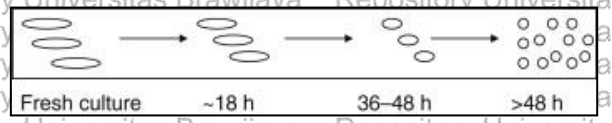
reducing the downstream processing costs required to dispose of process waste (Ritala et al., 2017). Bacteria that can produce SCP include: *Brevibacterium* sp, *Methylophilus* sp, *Acromobacter delvaevate*, *Acinetobacter calcoacenticu*, *Aeromonas hydrophilla*, *Bacillus* sp, *Lactobacillus* sp, *Cellulomonas* sp, *Methylomonas* sp, *Pseudomonas* sp, *Rhodopseudomonas* sp, *Flavobacterium* sp. The fermentation process using *Corynebacterium glutamicum* and *Brevibacterium lactofermentum* with molasses or starch hydrolyzate is the main method used for lysine production. The production of SPT uses waste material as a substrate, providing an economically viable protein source as animal feed and a product for human consumption as it meets dietary requirements for protein. (Kuhad et al., 1997; Wang et al., 2021).

SCP is always available in sufficient quantities because its production does not depend on the season. One of the local raw materials used in the manufacture of SCP is the waste product of Monosodium Glutamate. Most of the MSG waste consists of ammonium (NH4+) and polysaccharides, because the raw material for the manufacture of MSG is molasses which belongs to the carbohydrate group. Colloids derived from polysaccharides have a negative charge.

2.2. *Brevibacterium*

Brevibacterium is a genus of bacteria from the order *Actinomycetales*. They are gram-positive soil organisms. Obligate aerobic organisms are nonmotile, do not form spores, and not acidic quickly. *Brevibacterium* can grow at a temperature of 4 - 42^{o}C and optimum at a temperature of 21 – 28^{o}C. Produces stems in singlets, pairs, or short chains ranging

from 0.6 to 2.51 µm. Over time, about 2 days, the stems change to cocci at about 0.6 – 1 µm. The rods dominate in the exponential phase and turn into cocci in the stationary phase (Figure 2). Changes in cellular morphology related to methionine concentration, pH of the growing medium, growth temperature, and aeration. *Brevibacterium* also reduces nitrates to nitrites, along with being lipase positive, urease negative, oxidase variable, catalase positive, litmus milk positive, and DNAse (Forquin and Weiner, 2014).



Source: Encyclopedia of Food Microbiology (2014)

Figure 2. Cellular morphology change during growth

Forquin and Weiner (2014) revealed that Brevibacteria grow in a wide pH range, starting from pH 5.5 and continuing up to 10 with an optimum value of 7.0. As the salt concentration increases, the ability of organisms to grow at lower pH will decrease, but these organisms often produce large amounts of ammonia to raise the pH above 7,0. According to Jones and Keddie (1986); Franciscon et al., (2012) *Brevibacterium* is gram-positive chemoorganotrophic, obligate aerobic and halotolerant to halophiles (usually grows well at 8% NaCl with some being able to grow at 15% NaCl), and is found in a variety of habitats, especially those with high salt concentrations.

Brevibacterium is one of the most widely used corynebacterial in the fermentation industry, and has been widely used in the production of L-valine, L-glutamate, L-

threonine and L-lysine (Xu et al., 2011). Javed et al., (2011) revealed that this bacterial strain would be an ideal source for obtaining essential supplements compared to conventional protein sources. The genus *brevibacterium* is capable of metabolizing many different carbon and nitrogen sources for growth, with acetate and lactate overriding very common substrates for growth. This species also uses glucose and galactose as carbon sources (Forquin and Wainer, 2014), while sucrose and lactose have not been proven to be good carbon sources for brevibacterium culture (Javed et al., 2011). In the presence of lactic acid as the sole carbon source, Brevibacteria can utilize ammonium sulfate as a source of inorganic nitrogen and sulfur.

2.3. Broiler Nutritional Needs

Broiler are very efficient in converting feed into meat. Chicken will consume feed to meet their energy needs, and will continue to eat until their energy needs are met. The lower the energy content in the feed, the more broiler will consume the feed. Conversely, if the energy in the feed is high, the chicken will consume less feed. Fitasari (2012) states that the formulation of broiler feed needs to pay attention to the balance of energy and amino acids, where the addition of enzymes in the feed, especially proteases and amylase to increase the digestibility of protein and carbohydrates.

Table 1. Broiler nutritional needs for starter and finisher

No.	Parameter	Period	
		Starter	Finisher
1	Water Content	Max. 14,00 %	Max. 14,00 %
2	Crude Protein	Min. 20,0 %	Min. 19,0 %
3	Crude Fiber	Max. 5,0 %	Max 6,00 %
4	Crude Fat	Max 5 %	Min 5,00 %
5	Ash	Max. 5,0 %	Max. 8,00 %
6	Calsium	0,80 – 1,10 %	0,80 – 1,10 %
7	Total Phosphor	Min 0,50 %	Min 0,45 %
8	Available Phosphorus	Min. 0,60 %	Min. 0,55 %
9	Total Aflatoxin	Max 50,0 µg/Kg	Max 50,00 µg/Kg
10	Metabolic Energy	Min. 3000 kkal/kg	Min. 3100 kcal/kg
11	Amino Acid		
	Lysine	Min. 1,20 %	Min. 1,05 %
	Methionine	Min. 0,45 %	Min. 0,40 %
	Methionine + Cystine	Min. 0,80 %	Min. 0,75 %
	Threonine	Min 0,75 %	Min 0,65 %
	Tryptophan	Min 0,19	Min 0,18 %

Source: SNI (2015)

2.4. Commercial feed

Commercial feed is feed designed to produce optimal growth, health and appearance because it has been prepared based on the nutritional needs of livestock from a complete and quality nutritional content, but in commercial feed antibiotics are used as one of the feed additives (Sitompul et al., 2016). Feed is the main factor in determining the success of broiler rearing. Feed takes up 60-80% of the total production cost. Protein is one of the important elements in feed that is needed

for growth and efficiency of poultry feed. According to Ardianto (2018), feed is a formulation of various feed ingredients that are formulated with certain limitations so as to produce a formula containing the desired nutrients. The use of feed will greatly affect the performance of broiler. Feeding management must also be considered in order to reduce production costs but with optimal production results.

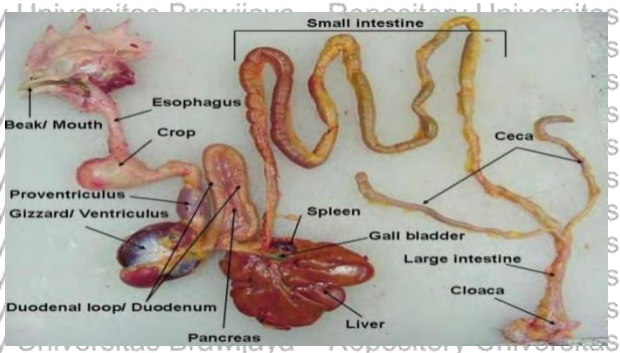
2.5. Broiler

Broiler belong to the order *Galliformes*, family *Phasianidae* and subspecies *Gallus domesticus*. Broiler have economic characteristics with the characteristics of fast growth, large body size, calm temperament, feathers close to the body and white, white skin, and low egg production. The weakness of broiler is that they are more sensitive to infectious diseases. So that broiler maintenance is very necessary for good environmental sanitation (Ariska, 2012). The superiority of broiler is supported by genetics and environmental conditions such as feed, ambient temperature, and maintenance management. Feed has a close relationship with body weight gain. With increasing body weight, it can be seen the ability of broiler to digest feed.

Broiler is a type of poultry that has superior potential from crosses of chicken breeds that have high productivity. Soffa (2012) stated that broiler are types of broiler that have been undergoing breeding efforts for a long time, so that they have a uniform shape, size and color. With this breeding effort, broiler is intended to produce meat and are economically profitable. With a short time of rearing in 4-5 weeks, broiler can be harvested weighing 1.5 – 2.2 kg.

2.6. Digestive Tract System

The digestive system of broiler has an important role in processing food into energy that will be used in daily livestock activities. The important organs that make up the digestive system are the beak, esophagus, crop, proventriculus, gizzard, small intestine, ceca, rectum, and cloaca. The digestive organs of broiler can be seen in Figure 3.



Source: Rahayu (2020)

Figure 3. Poultry digestive tract

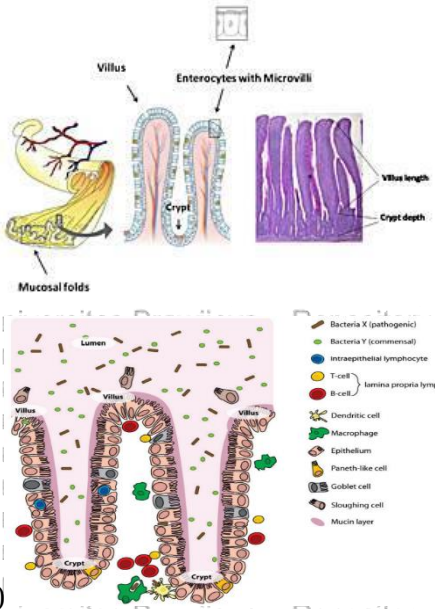
Rahayu (2020) states, the digestive tract of broiler consists of three types of digestion, namely (1) mechanical/physical digestion, carried out by the gizzard, (2) chemical/enzymatic digestion, carried out by digestive enzymes produced by the salivary glands in the mouth (amylase), proventriculus and gizzard (pepsin and lipase), duodenum (trypsin amylase, collagenase, bile salts, and lipase), jejunum (maltase, sucrase, lactase, peptidase), which functions to break protein, fat and carbohydrate bonds, and (3) Microbiological digestion, digestion that occurs in the cecum and colon.

Digestion of feed starts from the beak, continues in the mouth, which contains salivary glands with low concentrations of enzymes. The esophagus is a flexible tube that connects the mouth with the crop, serving as a digester and temporary food storage. Towards the proventriculus, which is a glandular stomach, chemical digestion occurs. The more phytic acid and crude fiber in the feed will increase the size of the proventriculus because it must produce HCl and pepsin to break down protein and phosphorus in the feed. The ventriculus (gizzard) is chemically and mechanically composed of two sets of strong muscles that act as teeth, containing materials such as grit, coral, and gravel that aid in grinding. The feed that enters the gizzard is ground and ground with liquid (an enzyme secreted by the proventriculus).

Feed pushed into the small intestine, the organ where it digests and breaks down food into smaller pieces so that it is easily absorbed by the body. Digestion occurs in the duodenum, while absorption occurs in the jejunum and ileum for this purpose, the duodenum receives digestive enzymes from the pancreas, namely amylase, lipase, and protease (Rahayu, 2020). Digestion that occurs in the duodenum is the breakdown of nutrients in the form of starch, fat and protein and secretes the enzymes trypsin, amylase, and lipase from the pancreas and bile from the liver to digest feed. Next to Jejunum which is the largest absorption of feed substances in the body of the chicken. In the ileum there are many microvilli that function to absorb digestive products. In the cecum or commonly called the appendix, digestion occurs microbiologically by microbes that help digestion.

The inner surface of the small intestine is uneven, but has mucosal folds that increase its surface area and aid in

mixing ingesta. The mucosa forms the intestinal villi – small finger-like protrusions that increase the surface and absorption area of the intestinal wall, providing efficient absorption of nutrients from the lumen. The crypts are trench-like invaginations of the epithelium around the villi. Towards the base of the crypts are stem cells, which continue to divide and provide the source of all epithelial cells in the crypts and in the villi (Mohnl, 2011). Cross-sectional illustration of the small intestine can be seen in Figure 4.



Source: (i) Mohnl (2011); Schokker (201)

Figure 4. Small intestine cross section

2.6.1. pH of Small Intestine

Emma et al., (2013) stated that the normal digesta pH in each part of the small intestine is

different, in the duodenum pH 5-6, jejunum pH 6.5-7 and ileum pH 7-7.5. Acidic conditions of the small intestine will reduce the growth of pathogenic bacteria, so that it can improve the condition of the digestive tract and nutrient digestibility which causes the feed rate in the small intestine to be better in the process of nutrient absorption (Rahmawati et al., 2014). Microbial balance in the digestive tract is influenced by pH, humidity, body temperature, environment, feed and contamination of excreta.

2.6.2. Viscosity of Small Intestine Digesta

Viscosity determines the rate of digesta in the digestive tract of livestock. Poultry has a relatively faster digesta song because the digestive tract of poultry is short. Sjofjan et al (2015) revealed that viscosity is the resistance to flow from a system caused by shear. The higher the crude fiber content, the faster the digesta rate, the faster the digesta rate, the shorter the digestive process in the digestive tract. In short, the digestive process results in less time for enzymes to completely degrade nutrients.

2.6.3. Digestive Enzymes

Effendy (2014) states that enzymes are protein molecules that act as biocatalysts and function to catalyze metabolic reactions that take place in organism. This function is influenced by environmental factors such as temperature, acidity (pH), substrate concentration, enzyme and activator concentration. Enzymes work in small amounts to

help break down complex organic compounds into simpler ones without changing the structure of the reaction. The rate of enzymatic reaction will increase with increasing enzyme concentration. Enzymes speed up chemical reactions by lowering their activity energy.

Proteins, starch and triglycerides, are the main macromolecules in food which are hydrolyzed by the respective pancreatic enzymes, namely proteases (trypsin and chymotrypsin), amylase and lipase. Enzymes from the small intestine mucosa play an important role in the digestive process, including disaccharides, aminopeptidase, phosphate, amylase and lipase. These enzymes can be produced alone or by microbes found in the digestive tract. Amylase is a group of catalytic enzymes that function to hydrolyze sugar and starch. Amylase digests carbohydrates (polysaccharides) into smaller disaccharide units and converts them into monosaccharides such as glucose (Sugiantoningsih, 2012). Protease enzymes can play a role in the digestive process, especially the breakdown of protein into amino acids (Effendy, 2014). Amino acids are absorbed by the chicken body which will then be converted into body protein. This is in accordance with Fitasari (2011) amino acids are needed by broiler in the formation of meat.

2.7. Excreta Ammonia Level

Broiler do not have a mechanism for amino acid storage, so excess amino acids are then excreted in the urine as uric acid (80%), ammonia (10%), and urea (5%).

Microorganism activity in broiler manure decomposes waste products such as unabsorbed protein, amino acids, other Non-Protein Nitrogen (NPN) compounds to form ammonia gas (NH3), hydrogen sulfide (H2S), nitrate, and nitrite (Goldstein and Skadhauge, 2000); Andinni, 2021). NH3 gas can reduce the appearance of livestock, and increase the possibility of disease in livestock and pollute the environment. The higher the ammonia indicates the absorption of nutrients is not efficient.

Ammonia is a gas resulting from the decomposition of nitrogenous waste materials in excreta, such as uric acid, unabsorbed protein, amino acids and other non-protein nitrogen (NPN) compounds due to the activity of microorganisms in feces (Manin et al., 2010; Riza et al., 2015). The smell in the cage environment is caused by the gases produced. Ammonia gas also plays a very important role in the health status, productivity level and performance of poultry and livestock health in cages. Ammonia levels with levels >25 ppm can cause cilia damage and susceptible to diseases such as New Castle Diseases (ND) (Riza et al, 2015). NH3 levels in the cage should not be more than 25 ppm and the threshold level for humans is 25 ppm for 8-10 hours. Tolerance limits for ammonia levels in broiler are presented in Table 2.

Table 2. Tolerance limits for ammonia levels in broiler

NH3 Level (ppm)	Effect
20	Disrupt the health and performance of broiler, increase in tetelo disease (New Castle Disease / ND) and damage to the respiratory system (in a long time).
25	Low body weight gain, decreased feed efficiency (for 42 days), causing airsacculitis followed by infectious bursal disease (after 56 days)
25 - 125	Decreased feed consumption and feed efficiency, causing symptoms of poisoning in broiler including irritation of the trachea, inflammation of the air sacs, conjunctivitis, and dyspnea
75 - 100	Respiratory epithelial changes, including loss of cilia and increased number of mucus-secreting cells
46 - 102	Causes damage to the eye in the form of keratoconjunctivitis

Source: Riza et al., (2015)

CHAPTER III
RESEARCH MATERIAL AND METHODS

3.1. Research location and time

This research was held on September 25th to November 14th, 2021 at the Field Laboratory Sumbersekar of the Faculty of Animal Science, Universitas Brawijaya Sumber Sekar, Dau, Malang as a place of rearing. Analysis of pH, viscosity of small intestine digesta and levels of excreta ammonia were carried out in the Laboratory of Nutrition and Animal Feed, Faculty of Animal Science, Universitas Brawijaya. Analysis of enzyme activity (amylase and protease) of small intestine digesta was carried out at the Biochemistry Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Brawijaya.

3.2. Research materials

3.2.1. Broiler

The livestock used were 480 DOCs that were unsexed and were reared for 35 days. The average weight of DOC was 47.42 ± 1.86 g/head and the coefficient of variation was 3.92%. Complete data can be seen in Appendix 1.

3.2.2. Pen and equipment

The cages used are made of bamboo with a litter cage system from rice husks. The pen will be separated so that there are 30 units with a size (length x width x height) of 150 x 120 x 70 cm and each pen is filled with 16 broilers. The floor plan of the cage

can be seen in Figure 5. The following are the equipment and supplies in the study:

a. 30 units feed and drink containers made of plastic are placed in each unit pen
b. 30 of 5-watt incandescent bulbs are used as lighting and heating in each enclosure unit and 2 of 50-watt bulbs are used as lighting
c. Gasolek used as a warmer during the brooder period (1-14 days)
d. A chamber thermohygrometer is used to measure temperature and humidity in the cage
e. Sanitary/cleaning equipment such as spray, disinfectant, and soap as well as brooms
f. Digital and hanging scales used for weighing feed and broiler
g. Equipment for cutting chicken, namely cutting knife, medium basin, bucket, plastic
h. Film pots were used as small intestine digesta sample containers, and plastic clips as excreta sample containers
i. Analysis equipment for pH (ph Meter), viscosity (Viscometer) and ammonia levels (Conway's cup), and enzyme activity.

P1U5	P4U6	P4U4	P3U3		P2U5	P2U1	P0U2
P4U1	P3U2	P3U1	P4U1		P0U3	P0U6	P2U4
Gasolec				pathway			Gasolec
P1U1	P1U6	P4U5	P0U5		P2U6	P3U5	P1U4	P1U2
P2U3	P1U3	P3U4	P2U2		P3U6	P0U4	P0U1	P4U2

Figure 5. Research cage plan

3.2.3. Organik protein

The Organik protein used is a by-product of processing food flavoring MSG fermented with brevibacterium. Organik protein is a product that produce by PT. Miwon Indonesia in a liquid form. The content of Organik protein can be seen in Table 3.

Table 3. Organik protein content

Content	Liquid Organik protein
Moisture (%)	44,94
Ash (%)	4,80
Crude Protein (%)	40,10
Crude Fat (%)	0,30
Crude Fiber (%)	0,07
Carbohydrate (%)	9,79
Caloric Value (kcal/100 g)	202,26

Source: PT. Miwon Indoensia

3.2.4. Feed and Composition of Feed Ingredients

The basal feed used was commercial feed and added Organik protein according to the level of use as a treatment. Feed is given twice a day, in the morning and evening, and drinking water is provided ad libitum. The treatment feed was mixed one day before feeding. The way of mixing feed is the OP poured into basal feed and then stirred manually by hand. The treatment feed was given starting from DOC until 35 days of sampling. The nutritional content of BR1 and

BR2 can be seen in Table 4, while the content of the treated feed substances can be seen in Table 5.

Table 4. Content of BR1 and BR2

Nutrition Content	BR1 (Starter)	BR2 (Finisher)
Water Content (%)	12	12
Ash (%)	7	7
Protein (%)	21	19
Crude Fat (%)	5	3 – 8
Crude Fiber (%)	5	5

Source: PT. Japfa Comfeed TBK

Table 5. Content of the treated feed substances

	Dry Content (%)	Crude Protein (%)	Crude Fat (%)	Crude Fiber (%)	Ash (%)	EM (kcal/kg)
			Starter			
P0	89,89	22,44	6,37	8,07	4,98	2914,8
P1	89,33	23,71	6,02	4,83	5,15	2889,6
P2	88,43	23,04	5,41	5,13	5,35	2828
P3	86,10	23,43	5,47	4,59	5,35	2816,1
P4	89,20	23,40	5,89	4,60	5,53	277,6
			Finisher			
P0	90,45	18,10	-	-	-	-
P1	89,20	18,71	-	-	-	-
P2	88,98	18,90	-	-	-	-
P3	88,61	19,43	-	-	-	-
P4	87,65	20,43	-	-	-	-

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

3.3. Research Methods

Research on the use of Organik protein was carried out using a field experiment method using a completely randomized design (CRD) with 5 treatments and 6 replications so that there were 30 experimental units. Each unit consisted of 16 broilers and a total of 480 broilers used in this study. The treatment feed was given starting on the first day until the 35th day. The feed treatments studied were:

P0 = basal feed 100%
P1 = basal feed 98,5 %+ Organik protein 1,5 %
P2 = basal feed 97 % + Organik protein 3 %
P3 = basal feed 95,5 % + Organik protein 4,5 %
P4 = basal feed 94 % + Organik protein 6 %

3.4. Research Procedures

3.4.1. Cages Preparations

Preparation of the cage was carried out 3 weeks before the DOC arrived, including making the cage unit and sanitizing the cage and equipment to be used. Sanitize the cage by spraying an antiseptic disinfectant throughout the cage. The cages were divided into 30 plots, each plot measuring 150 x 120 x 70 cm at the age of 1-14 days and 240 x 150 x 70 cm at the age of 15-35 days. Each unit of the cage was given a feeder, a drinking place, and a 5-watt incandescent lamp and was given a treatment code and replication. Gasolec is placed on the edge of the cage as a heater. The cage was given a pad of husks and

newspapers. Newspaper mats are only given for the first 7 days of DOC chick in. After not being given a newspaper, the husk is added once every 4 days or if it's wet.

3.4.2. Rearing

Rearing carried out for 35 days. DOC was weighed when chick in and randomly placed into the pen. The first 3 hours after weighing, DOC was given brown sugar water as the first energy intake and post-travel stress management. During brooding, gasolec is turned on 24 hours for 2 weeks. Check feed, drinking water, and cage conditions every morning and evening. Feed is given in the morning and evening, drinking water is provided ad libitum. The feeding treatment was carried out from the first day according to the treatment. Weighing the remaining feed and body weight every seven days.

Rearing with a metabolic cage was carried out for 3 last days for the collection of excreta ammonia samples. Each metabolic cage unit is filled with one chicken. Feed treatment in the morning and evening and drinking water ad libitum. Weighed feed and feed residue every day.

3.4.3. Sampling

Sampling was carried out at the end of rearing on the 35th day. Broiler are slaughtered by cutting off three channels, namely the respiratory tract, digestive tract and jugular vein. After that, the hair is removed after being dipped in hot water. The organs from the

small intestine were taken, the contents were removed and stored in a film pot and then stored in the freezer until the time for testing in the laboratory.

The excreta were collected every morning during metabolic maintenance, weighed, sprayed with formalin and boric acid to bind N to prevent evaporation. The excreta were placed in plastic and coded according to treatment, then dried in the sun for 5 days. Furthermore, the excreta samples were dried again in an oven at 60oC for 24 hours.

3.4.4. Test of pH Small Intestine Digesta

Preparation of tools and materials needed. A sample of 1 gram of small intestine digesta was weighed and then distilled water was added to a volume of 10 ml, then centrifuged at 2500 rpm for 10 minutes. The pH meter was calibrated with a buffer solution of pH 4 and then wiped with a dry tissue. Put the pH meter into the distilled water and then wipe it again with a dry tissue. After that the pH meter is inserted into the sample. Note the numbers listed on the pH meter.

3.4.5. Test of Viscosity Small Intestine Digesta

Preparation of tools and materials needed. A sample of 1 gram of small intestine digesta was weighed and then distilled water was added to a volume of 10 ml. then centrifuged at 2500 rpm for 10 minutes. The supernatant liquid was taken, then the viscosity was measured using a Digital Viscometer

NDJ-5S. Tested by determining the rotor and rotor speed. Recorded the results on the monitor.

3.4.6. Test of Amylase Enzyme Activity

Enzyme activity was analyzed using the method of Nelson Somogyi (1952). The Somogyi-Nelson method is a method of determining the content of reducing sugars, where in principle, reducing sugar will reduce Cu2+ ions to Cu+ ions, then these Cu+ ions will reduce arsenomolybdate compounds to form a blue-green complex (Nelson, 1952).

Prepared test tubes and given a table for each treatment code as well as control tubes and blank tubes. The test tube was put into a thermostat water bath at 50oC and left for 5 minutes. Then, 0.5 mL of enzim amylase was added to each tube, the incubation was continued for 30 minutes. Next, the test tubes were removed and 1 mL of Nelson-Somogyi reagent was added each and 0.5 mL of substrate was added to the control tube and 1 mL of 0.05 M phosphate buffer pH 7 was added, then all tubes were vortexed. Furthermore, the mouth of the test tube was closed with marbles and put into a heater filled with hot water and left for ± 20 minutes. Then it was cooled to room temperature and into each test tube, 1 mL of arsenomolybdate solution and 6 mL of 0.05 M phosphate buffer pH 7 were added, then vortexed and allowed to stand again for 30 minutes. The absorbance of each solution was measured with a UV-Vis spectrophotometer at a wavelength of 540 nm. Each test sample was replicated once.

3.4.7. Test of Protease Enzyme Activity **(Bergemeyer and Grassl, 1983)**

A total of 0.1 mL of enzyme solution was added with 0.1 mL of 0.05 M phosphate buffer pH 7, preincubated at 37°C for 5 minutes. Then 0.1 mL of substrate (2% casein in 0.05 M phosphate buffer solution pH 7) was added, incubated at 37°C for 10 minutes. The reaction was stopped by adding 0.2 mL of 0.4 M trichloroacetic acid (TCA) and centrifuged. A total of 0.2 mL of the centrifuged filtrate was added with 1 mL of 0.5 M sodium carbonate, preincubated for 10 minutes, and then 1 mL of ninhydrin reagent was added and allowed to stand for 30 minutes. The absorbance was measured using a spectrophotometer at a wavelength of 578 nm. The proteolytic activity of the enzyme was calculated by the formula.

Protease Activity: $\frac{tyrosine \times V}{(p \times q)Fp}$

With:

Tyrosine : tyrosine concentration formed
v : total volume of sample in each tube
q : incubation time
p : total enzyme (mL)
Fp : dilution factor

3.4.8. Test of Excreta Ammonia Level

The measurement of ammonia (NH3) production was carried out using the Conway microdiffusion technique, first the edges of the Conway cup and the lid were smeared with vaseline. Take 1.0 ml of the supernatant and place it on one end

of the groove in a Conway cup that has been smeared with vaseline on the lips and lid. 1.0 ml of saturated Na2CO was placed at one end of the other cup next to the supernatant (the two solutions should not be mixed before being tightly closed). 1.0 mL of the indicator boric acid solution was placed in a small cup located in the center of the Conway dish. Conway cup tightly closed. Mix the Na2CO solution with the supernatant until evenly distributed. Wait for 24 hours at room temperature. Boric acid is titrated with 0.005 N H2SO4 on a magnetic stirrer. The NH3 content of the H2SO4 titration was calculated using the formula: NH3 level = volume of H2SO4 (ml) x N H2SO4 x 100

3.5. Research Variable

a. pH of small intestine digesta
b. viscosity of small intestine digesta (CPs)
c. Amylase enzyme activity of small intestine (µmol /g minute)
d. Protease enzyme activity of small intestine (µmol /g minute)
e. Excreta ammonia level (ppm)

3.6. Statistics Analysis

Data from the research results were recorded and calculated using the Microsoft Excel program. Furthermore, the data were analyzed using ANOVA from a Completely Randomized Design (CRD). If the results are significantly different ($P<0.05$) or highly significant different ($P<0.01$), it is continued with Duncan's Multiple Distance Test (DMRT) to determine the difference effect between treatments.

Completely Randomized Design mathematical model as follows:

$$Yij = \mu + \tau i + \varepsilon ij$$

With:

Yij : value of observations to be analyzed
μ : general mean value
τi : effect of treatment using Organik protein level to-i
εij : Error treatment
i : 1, 2, 3, 4, 5
j : 1, 2, 3, 4, 5, 6

3.7. Terminology and Discussing Limitation

a. DOC is a one day old chick
b. Ad libitum is the provision of food or drink that is always available
c. OP (Organik protein) is a by-product of animal feed processing MSG (Monosodium Glutamate) from the production of PT. MIWON Indonesia
d. SCP (Single Cell Protein) is a component of feed ingredients in Organik protein with a fairly high protein content (40%) and contains a fairly complete range of amino acids.
e. pH is a measure of the potential for hydrogen to measure the acidity of a material
f. Viscosity is a measure of the viscosity of the fluid which states the size of the friction in the fluid.

CHAPTER IV
RESULTS AND DISCUSSIONS

Based on the results of research on the use of Organik protein in feed on pH, viscosity, and enzyme activity (amylase and protease) of the small intestine and levels of excreta ammonia in broiler, it can be seen in Table 6.

Table 6. Average value of treatment on pH, viscosity, and enzymes activity of small intestine and excreta ammonia levels in broiler.

	pH Small Intestine Digesta	Viscosity of Small Intestine Digesta (CPs)	Enzyme Amylase (µmol /g minute)	Enzyme Protease (µmol /g minute)	Ammonia Level (ppm)
P0	6,78 ± 0,50	3,33 ± 0,82	549,97±25,02^{BC}	5,21 ± 0,02^{C}	0,118 ± 0,13
P1	6,87± 0,37	3,17 ± 0,75	502,19±11,86^{C}	4,99 ± 0,54^{CD}	0,233 ± 0,11
P2	6,90 ± 0,49	3,17 ± 0,41	670,825±47,64^{A}	3,95 ± 0,40^{D}	0,238 ± 0,24
P3	6,92 ± 0,29	3,08 ± 0,66	581,67±91,03^{B}	6,62 ± 1,18^{B}	0,34 ± 0,15
P4	6,68 ± 0,33	3,50 ± 0,55	547,81±50,08^{C}	7,89 ± 0,08^{A}	0,40 ± 0,14

With: Value with different superscript letters (A-D) in the same column showed a highly significant different effect (P<0,01).

4.1. The Effect of Treatment on the pH of the Small Intestine Digesta

The complete data on the effect of treatment on pH of the small intestine digesta of broiler can be seen in Table 6.

The average value of the pH test results of the small intestine digesta from the lowest to the highest is treatment P4 (6,68 ± 0,33), P0 (6,78 ± 0,5), P1 (6,87 ± 0,37), P2 (6,9 ± 0,49), P3 (6,92 ± 0,29). Data and results of analysis of various degrees of pH small intestine digesta of broiler with the use of Organik protein can be seen in Appendix 2. Intestinal digesta used in this study was taken from the ileum of the small intestine of broiler.

The results of the analysis of variance showed that the use of Organik protein in feed had no significant different effect (P>0,05) on pH of small intestine digesta of broiler. The lowest value was in treatment P4 (6.68 ± 0.33) with the use of Organik protein as much as 6%, and the highest value at P3 (6.92 92 ± 0.29) with the use of Organik protein as much as 4.5%. The pH value of the small intestine of broiler according to Gauthier (2007) and NRC (2012) can be seen in Table 7.

Table 7. pH of small intestine digesta broiler according to Gauthier and NRC

Small Intestine	pH [a]	pH [b]
Duodenum	5,0 – 6,0	5,70 – 6,00
Jejenum	6,5 – 7,0	5,80 – 5,90
Ileum	7,0 – 7,5	6,30 – 6,40

Source: a. Gauthier (2007)
b. NRC (2012)

The acidity of the digestive tract parts has an influence on the life of digestive microbes which are closely related to the products of digestive enzymes and enzymes from microorganism products from feed (Sjofjan et al, 2020). Gastric acid (HCl) will be released by digestive juices

naturally to form acidic conditions. Stomach acid conditions serve as a selection of microbes that will enter the intestine.

The pH level in the intestinal area is a factor that forms 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. In contrast, beneficial microorganisms live at lower pH and compete with pathogens. Decreasing the pH of the digestive tract, especially the small intestine, 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, so that it can improve the condition of the digestive tract and nutrient digestibility which causes the feed rate in the small intestine to be better in the process of nutrient absorption (Puspasari et al., 2016).

4.2. The Effect of Treatment on the Viscosity of the Small Intestine Digesta

The complete data on the effect of treatment on the viscosity of the small intestine digesta of broiler can be seen in Table 6. The average value of the viscosity test results (CPs) of the small intestine digesta from the lowest to the highest, respectively, is treatment P3 (3,08 ± 0,66), P2 (3,17 ± 0,41), P1 (3,17 ± 0,75), P0 (3,33 ± 0,82), P4 (3,50 ± 0,55). Data and results of analysis of various viscosity of small intestine digesta of broiler with treatment using Organik protein can be seen in Appendix 3.

The results of the analysis of variance showed that the use of Organik protein had no significantly different effect (P>0,05) on the digesta viscosity of the broiler small intestine.

Low viscosity indicates the nature of amino acids (the result of protein hydrolysis) which are soluble in water (Fitasari, 2011). The negative effect if the viscosity of the contents of the small intestine increases is to reduce the efficiency of digestion by slowing the rate of diffusion of endogenous enzymes to react with substrates and nutrients and compressing absorption in the villi in the small intestine wall (Natsir et al., 2016). However, Rosningsih and Sundari (2015) stated that the increased digesta viscosity resulted in a slower digesta rate and allowed an increase in the digestive process and more effective nutrient absorption, so that the availability of nutrients for the synthesis of body tissues increased.

Sjofjan et al., (2015) revealed that viscosity is the resistance to flow from a system caused by shear. The greater the resistance or shear, the more viscous the system. According to Kusumaningtyaswati (2018), viscosity is influenced by temperature, pressure, weight, solution molecules, solution concentration and dissolved materials present. Viscosity determines the rate of digestion of the digestive tract. Poultry has a relatively faster digesta rate because the digestive tract of poultry is short. Increased ileal digesta viscosity can affect nutrient absorption and also affect intestinal motility. Widodo (2010) also 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.

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. Increased viscosity indicates the chyme in the intestine is denser, so absorption is not optimal. Research by Cahyaningsih

et al., (2013) stated that the addition of lactic acid bacteria in feed causes the atmosphere in the digestive tract to be more acidic and the pH in the small intestine decreases, the viscosity of the digesta increases so that the rate of digestion runs slower and improves digestion in the stomach and intestines.

4.3. The effect of Treatment on the Amylase Enzyme Activity of the Small Intestine

The complete data on the effect of treatment on the activity of the amylase enzyme in the small intestine of broiler can be seen in Table 6. There was a decrease in the value of the amylase enzyme activity in the use of OP at the level of 3% and 6% when compared to the control treatment (P0). The average value of the intestinal enzyme activity test results (μmol/ g) from the lowest to the highest, respectively, were treatments P1 (502,19 ± 11,86), P4 (547,81 ± 50,08), P0 (549,97 ± 25,21), P3 (581,67 ± 91,03) and P2 (670,83 ± 47,64). Data and results of analysis of various viscosity of small intestine digesta of broiler with treatment using Organik protein can be seen in Appendix 4.

The results of analysis of variance showed that the use of Organik protein had highly significant different effect ($P<0,01$) on amylase enzyme activity. To determine the difference between treatments, the DMRT test was carried out. Based on this test, it was found that in the use of OP 3% (P2) significantly different to all treatment. P3 was not significantly different to P0 and different to other treatment, while P1 has the same effect to P4 as well as not significantly different to P0.

The decrease in amylase enzyme activity at the use of 1.5% and 6% of the control feed comparison was thought to

have occurred due to the presence of inhibitory factors in the OP that affected enzyme activity. Sugiantoningsih (2012) states that the work of enzyme activities can be influenced by temperature, pH, concentration of enzymes, substrates, cofactors and enzyme inhibitors. At the optimum pH, the enzyme can decompose the substrate maximally. Amylase is an enzyme that digests polysaccharides and starch which is a complex food ingredient which is then converted into simpler food substances, namely glucose which is a source of energy (Lehninger, 1994; Nurhayatin, 2016).

Cholidah (2011) revealed that single cell proteins are high in protein content and have high nucleic acids as well. Nucleic acids are macromolecules that have low digestibility when compared to pure protein (Samadi et al, 2012). It is known that nucleic acids can increase the activity of xanthine oxidase which plays a role in the formation of free radicals. The increase in free radicals causes the chicken's need for Se and Vitamin E to increase, so that the chicken is deficient in Se. Deficiency of Se and Vitamin E is associated with the use of SCP in animal feed, because some SCP have low Se content.

4.4. The Effect of Treatment on the Protease Enzyme Activity of the Small Intestine

The complete data on the effect of treatment on the amylase enzyme activity in the small intestine of broiler can be seen in Table 6. There was a decrease in the value of the amylase enzyme activity at P1 and P2 when compared to the control treatment P0. The average value of the protease enzyme activity test results (µmol/g) in the small intestine from the lowest to the highest, that is P4 (7,89 ± 0,08), P3

(6,62 ± 1,18), P0 (5,21 ± 0,02), P1 (4,99 ± 0,54) and P2 (3,95 ± 0,40). The data from the analysis of the viscosity variance of the digesta of the small intestine of broiler with the use of Organik protein can be seen in Appendix 5.

The results of the analysis of variance showed that the use of Organik protein had highly significant different effect (P<0,01) on the activity of the protease enzyme. To find out the differences between feeding treatments, the DMRT test was carried out (Appendix 5). Based on these tests, it was found that the use of OP 6% (P4) significantly different from all treatments as well as 4,5% (P3) and 3% (P2), while P0 not significantly different to P1. A highly significant different effect indicates that the use of Organik protein in feed can increase the activity of protease enzymes.

The activity of protease enzymes increased at the level of use of 4.5% and 6% of Organik protein. The increase in the value of enzyme activity is equivalent to Sugiantoningsih (2012) that single-cell protein fermented with brevibacterium can help increase the work of protease activity in the digestive tract during the process of absorption of food in the small intestine because it contains high protein and free amino acid compounds. According to Zainuddin et al., (1994); Primacitra et al., (2014) the level of protease enzyme activity is influenced by pH, concentration, temperature and substrate. High or low pH will reduce enzyme activity because most enzymes work optimally at neutral pH.

Protease enzymes play a role in the digestive process, especially the breakdown of protein into amino acids (Effendy, 2014). Amino acids are absorbed by the chicken body which will then be converted into body protein. according to Fitasari (2011) amino acids are indispensable for broiler in the

formation of meat. The reshuffle of amino acids is carried out by protein-breaking enzymes, namely proteases (Soffa, 2012). Organik protein is rich in protein, amino acids and vitamins which can be used as animal feed to compensate for amino acid deficiency. SCP has good quality and protein digestibility so that its use at a certain level can replace fish meal in feed (Ludfi, 2012).

4.5. The Effect of Treatment on the Excreta Ammonia Level

The complete data on the effect of treatment on the excreta ammonia levels of broiler can be seen in Table 6. There was an increase value of excreta ammonia levels at each additional level of protein organic use. The data and results of the analysis of variance in the levels of excreta ammonia of broiler with the use of Organik protein can be seen in Appendix 6. The results of the analysis of variance showed that the use of Organik proteins had no significant different effect (P>0,05) on the excreta ammonia levels of broiler.

The increase in ammonia value in the study was still within the normal limits of NH3 levels in broiler, which was below 20 ppm. Measurement of excreta ammonia levels can indicate whether the protein contained in the feed can be completely digested. The formation of ammonia in the body of livestock mainly occurs in caeca due to urease enzyme-producing bacteria that play a role in the hydrolysis of urea into ammonia (Karasawa et al., 1994; Hulu et al., 2019). The main factors that form ammonia are uricase which is produced by gram-negative bacteria and uric acid which is formed due to undigested protein in the digestive tract of broiler. The production of NH3 is closely related to the efficiency of

absorption of nutrients, especially proteins and amino acids. Protein that is not absorbed in the digestive tract will be converted into uric acid which is then excreted with feces (Hutauruk, 2017).

Excreta ammonia is an alkaline gas produced by livestock which is colorless and toxic, and has a high irritating power (Soma, 2016). The source of air pollution in chicken farms comes from chicken manure 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 ammonia, nitrate, and nitrite gases and sulfide gases. These gases are what cause the smell. The odor comes from the high content of ammonia gas and hydrogen sulfide (H2S) gas, dimethyl sulfide, carbon disulfide, and mercaptans. Hydrogen sulfide (H2S) is a gas that can produce an unpleasant odor (Nugrahani et al., 2016).

Ammonia is toxic to broiler and humans if it exceeds the threshold levels that can be tolerated by broiler and humans. Ammonia content is also influenced by environmental factors such as temperature and humidity. In addition, the environment, NH3 gas can reduce livestock, increase food for disease, and reduce work efficiency of human (Charles and Haryono, 1991; Riza et al., 2015). Ammonia levels with levels >25 ppm can cause ciliary damage to diseases such as News Castle Diseases (ND) causing a decrease in health status, performance level and productivity of poultry (Heij and Schneider, 1991; Riza at al., 2015).

CHAPTER V
CONCLUSIONS AND SUGGESTIONS

5.1. Conclusions

The use of Organik protein in feed can increase the activity of amylase and protease enzymes but does not decrease the pH and viscosity of the small intestine as well as the excreta ammonia level of broiler. The best treatment in this study was the use of 4.5% Organik protein level on enzyme activity.

5.2. Suggestions

Based on this research, it is recommended to use Organik protein as a protein source for broiler at a level of 4.5%. Mixing OP in feed by spraying so that the OP and basal feed are mixed more evenly. For further research, can be used of Organik protein in the form of flour to find out which form of Organik protein is better used.

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APPENDIXIES

Appendix 1. Body Weight of Broilers DOCs

Chicken number	DOC Weight (x)	Deviation $(X - \bar{x})$	Square of Deviation $(X - \bar{x})^2$
1	49,25	1,83	3,35
2	49,25	1,83	3,35
3	49,25	1,83	3,35
4	49,25	1,83	3,35
5	47,50	0,08	0,01
6	47,50	0,08	0,01
7	47,50	0,08	0,01
8	47,50	0,08	0,01
9	47,50	0,08	0,01
10	47,50	0,08	0,01
11	47,50	0,08	0,01
12	47,50	0,08	0,01
13	47,25	-0,17	0,03
14	47,25	-0,17	0,03
15	47,25	-0,17	0,03
16	47,25	-0,17	0,03
17	48,75	1,33	1,77
18	48,75	1,33	1,77
19	48,75	1,33	1,77
20	48,75	1,33	1,77
21	47,25	-0,17	0,03
22	47,25	-0,17	0,03
23	47,25	-0,17	0,03
24	47,25	-0,17	0,03
25	48,75	1,33	1,77

26	48,75	1,33	1,77
27	48,75	1,33	1,77
28	48,75	1,33	1,77
29	47,75	0,33	0,11
30	47,75	0,33	0,11
31	47,75	0,33	0,11
32	47,75	0,33	0,11
33	47,25	-0,17	0,03
34	47,25	-0,17	0,03
35	47,25	-0,17	0,03
36	47,25	-0,17	0,03
37	48	0,58	0,34
38	48	0,58	0,34
39	48	0,58	0,34
40	48	0,58	0,34
41	46,75	-0,67	0,45
42	46,75	-0,67	0,45
43	46,75	-0,67	0,45
44	46,75	-0,67	0,45
45	48,25	0,83	0,69
46	48,25	0,83	0,69
47	48,25	0,83	0,69
48	48,25	0,83	0,69
49	46	-1,42	2,02
50	46	-1,42	2,02
51	46	-1,42	2,02
52	46	-1,42	2,02
53	47	-0,42	0,18
54	47	-0,42	0,18
55	47	-0,42	0,18

56	47	-0,42	0,18
57	50,5	3,08	9,48
58	50,5	3,08	9,48
59	50,5	3,08	9,48
60	50,5	3,08	9,48
61	51,25	3,83	14,66
62	51,25	3,83	14,66
63	51,25	3,83	14,66
64	51,25	3,83	14,66
65	50,25	2,83	8,00
66	50,25	2,83	8,00
67	50,25	2,83	8,00
68	50,25	2,83	8,00
69	49,5	2,08	4,32
70	49,5	2,08	4,32
71	49,5	2,08	4,32
72	49,5	2,08	4,32
73	47,5	0,08	0,01
74	47,5	0,08	0,01
75	47,5	0,08	0,01
76	47,5	0,08	0,01
77	47,5	0,08	0,01
78	47,5	0,08	0,01
79	47,5	0,08	0,01
80	47,5	0,08	0,01
81	46,75	-0,67	0,45
82	46,75	-0,67	0,45
83	46,75	-0,67	0,45
84	46,75	-0,67	0,45
85	45	-2,42	5,86

86	45	-2,42	5,86
87	45	-2,42	5,86
88	45	-2,42	5,86
89	48,75	1,33	1,77
90	48,75	1,33	1,77
91	48,75	1,33	1,77
92	48,75	1,33	1,77
93	46,5	-0,92	0,85
94	46,5	-0,92	0,85
95	46,5	-0,92	0,85
96	46,5	-0,92	0,85
97	47,25	-0,17	0,03
98	47,25	-0,17	0,03
99	47,25	-0,17	0,03
100	47,25	-0,17	0,03
101	47	-0,42	0,18
102	47	-0,42	0,18
103	47	-0,42	0,18
104	47	-0,42	0,18
105	46,75	-0,67	0,45
106	46,75	-0,67	0,45
107	46,75	-0,67	0,45
108	46,75	-0,67	0,45
109	52,25	4,83	23,32
110	52,25	4,83	23,32
111	52,25	4,83	23,32
112	52,25	4,83	23,32
113	48,25	0,83	0,69
114	48,25	0,83	0,69
115	48,25	0,83	0,69

116	48,25	0,83	0,69
117	49,25	1,83	3,35
118	49,25	1,83	3,35
119	49,25	1,83	3,35
120	49,25	1,83	3,35
121	47,5	0,08	0,01
122	47,5	0,08	0,01
123	47,5	0,08	0,01
124	47,5	0,08	0,01
125	48,25	0,83	0,69
126	48,25	0,83	0,69
127	48,25	0,83	0,69
128	48,25	0,83	0,69
129	48	0,58	0,34
130	48	0,58	0,34
131	48	0,58	0,34
132	48	0,58	0,34
133	45,5	-1,92	3,69
134	45,5	-1,92	3,69
135	45,5	-1,92	3,69
136	45,5	-1,92	3,69
137	50,75	3,33	11,08
138	50,75	3,33	11,08
139	50,75	3,33	11,08
140	50,75	3,33	11,08
141	44,75	-2,67	7,13
142	44,75	-2,67	7,13
143	44,75	-2,67	7,13
144	44,75	-2,67	7,13
145	46	-1,42	2,02

146	46	-1,42	2,02
147	46	-1,42	2,02
148	46	-1,42	2,02
149	48	0,58	0,34
150	48	0,58	0,34
151	48	0,58	0,34
152	48	0,58	0,34
153	47	-0,42	0,18
154	47	-0,42	0,18
155	47	-0,42	0,18
156	47	-0,42	0,18
157	48,25	0,83	0,69
158	48,25	0,83	0,69
159	48,25	0,83	0,69
160	48,25	0,83	0,69
161	50,75	3,33	11,08
162	50,75	3,33	11,08
163	50,75	3,33	11,08
164	50,75	3,33	11,08
165	47	-0,42	0,18
166	47	-0,42	0,18
167	47	-0,42	0,18
168	47	-0,42	0,18
169	51,25	3,83	14,66
170	51,25	3,83	14,66
171	51,25	3,83	14,66
172	51,25	3,83	14,66
173	49,5	2,08	4,32
174	49,5	2,08	4,32
175	49,5	2,08	4,32

176	49,5	2,08	4,32
177	48	0,58	0,34
178	48	0,58	0,34
179	48	0,58	0,34
180	48	0,58	0,34
181	48,5	1,08	1,16
182	48,5	1,08	1,16
183	48,5	1,08	1,16
184	48,5	1,08	1,16
185	49,5	2,08	4,32
186	49,5	2,08	4,32
187	49,5	2,08	4,32
188	49,5	2,08	4,32
189	46,5	-0,92	0,85
190	46,5	-0,92	0,85
191	46,5	-0,92	0,85
192	46,5	-0,92	0,85
193	47,5	0,08	0,01
194	47,5	0,08	0,01
195	47,5	0,08	0,01
196	47,5	0,08	0,01
197	49,25	1,83	3,35
198	49,25	1,83	3,35
199	49,25	1,83	3,35
200	49,25	1,83	3,35
201	46,5	-0,92	0,85
202	46,5	-0,92	0,85
203	46,5	-0,92	0,85
204	46,5	-0,92	0,85
205	48	0,58	0,34

206	48	0,58	0,34
207	48	0,58	0,34
208	48	0,58	0,34
209	44,5	-2,92	8,53
210	44,5	-2,92	8,53
211	44,5	-2,92	8,53
212	44,5	-2,92	8,53
213	49	1,58	2,49
214	49	1,58	2,49
215	49	1,58	2,49
216	49	1,58	2,49
217	48	0,58	0,34
218	48	0,58	0,34
219	48	0,58	0,34
220	48	0,58	0,34
221	50,75	3,33	11,08
222	50,75	3,33	11,08
223	50,75	3,33	11,08
224	50,75	3,33	11,08
225	44	-3,42	11,70
226	44	-3,42	11,70
227	44	-3,42	11,70
228	44	-3,42	11,70
229	45,75	-1,67	2,79
230	45,75	-1,67	2,79
231	45,75	-1,67	2,79
232	45,75	-1,67	2,79
233	45,75	-1,67	2,79
234	45,75	-1,67	2,79
235	45,75	-1,67	2,79

236	45,75	-1,67	2,79
237	45,75	-1,67	2,79
238	45,75	-1,67	2,79
239	45,75	-1,67	2,79
240	45,75	-1,67	2,79
241	48,25	0,83	0,69
242	48,25	0,83	0,69
243	48,25	0,83	0,69
244	48,25	0,83	0,69
245	46,75	-0,67	0,45
246	46,75	-0,67	0,45
247	46,75	-0,67	0,45
248	46,75	-0,67	0,45
249	43,75	-3,67	13,48
250	43,75	-3,67	13,48
251	43,75	-3,67	13,48
252	43,75	-3,67	13,48
253	47	-0,42	0,18
254	47	-0,42	0,18
255	47	-0,42	0,18
256	47	-0,42	0,18
257	49,25	1,83	3,35
258	49,25	1,83	3,35
259	49,25	1,83	3,35
260	49,25	1,83	3,35
261	48,5	1,08	1,16
262	48,5	1,08	1,16
263	48,5	1,08	1,16
264	48,5	1,08	1,16
265	48,75	1,33	1,77

266	48,75	1,33	1,77
267	48,75	1,33	1,77
268	48,75	1,33	1,77
269	44	-3,42	11,70
270	44	-3,42	11,70
271	44	-3,42	11,70
272	44	-3,42	11,70
273	45	-2,42	5,86
274	45	-2,42	5,86
275	45	-2,42	5,86
276	45	-2,42	5,86
277	48,25	0,83	0,69
278	48,25	0,83	0,69
279	48,25	0,83	0,69
280	48,25	0,83	0,69
281	44,75	-2,67	7,13
282	44,75	-2,67	7,13
283	44,75	-2,67	7,13
284	44,75	-2,67	7,13
285	46,75	-0,67	0,45
286	46,75	-0,67	0,45
287	46,75	-0,67	0,45
288	46,75	-0,67	0,45
289	49,25	1,83	3,35
290	49,25	1,83	3,35
291	49,25	1,83	3,35
292	49,25	1,83	3,35
293	50	2,58	6,65
294	50	2,58	6,65
295	50	2,58	6,65

296	50	2,58	6,65
297	48,25	0,83	0,69
298	48,25	0,83	0,69
299	48,25	0,83	0,69
300	48,25	0,83	0,69
301	49,75	2,33	5,43
302	49,75	2,33	5,43
303	49,75	2,33	5,43
304	49,75	2,33	5,43
305	47,5	0,08	0,01
306	47,5	0,08	0,01
307	47,5	0,08	0,01
308	47,5	0,08	0,01
309	47,25	-0,17	0,03
310	47,25	-0,17	0,03
311	47,25	-0,17	0,03
312	47,25	-0,17	0,03
313	44,75	-2,67	7,13
314	44,75	-2,67	7,13
315	44,75	-2,67	7,13
316	44,75	-2,67	7,13
317	47	-0,42	0,18
318	47	-0,42	0,18
319	47	-0,42	0,18
320	47	-0,42	0,18
321	46,75	-0,67	0,45
322	46,75	-0,67	0,45
323	46,75	-0,67	0,45
324	46,75	-0,67	0,45
325	48	0,58	0,34

326	48	0,58	0,34
327	48	0,58	0,34
328	48	0,58	0,34
329	48	0,58	0,34
330	48	0,58	0,34
331	48	0,58	0,34
332	48	0,58	0,34
333	48	0,58	0,34
334	48	0,58	0,34
335	48	0,58	0,34
336	48	0,58	0,34
337	46,75	-0,67	0,45
338	46,75	-0,67	0,45
339	46,75	-0,67	0,45
340	46,75	-0,67	0,45
341	45,5	-1,92	3,69
342	45,5	-1,92	3,69
343	45,5	-1,92	3,69
344	45,5	-1,92	3,69
345	43,75	-3,67	13,48
346	43,75	-3,67	13,48
347	43,75	-3,67	13,48
348	43,75	-3,67	13,48
349	46,75	-0,67	0,45
350	46,75	-0,67	0,45
351	46,75	-0,67	0,45
352	46,75	-0,67	0,45
353	49	1,58	2,49
354	49	1,58	2,49
355	49	1,58	2,49

356	49	1,58	2,49
357	45,5	-1,92	3,69
358	45,5	-1,92	3,69
359	45,5	-1,92	3,69
360	45,5	-1,92	3,69
361	45,75	-1,67	2,79
362	45,75	-1,67	2,79
363	45,75	-1,67	2,79
364	45,75	-1,67	2,79
365	49	1,58	2,49
366	49	1,58	2,49
367	49	1,58	2,49
368	49	1,58	2,49
369	46,75	-0,67	0,45
370	46,75	-0,67	0,45
371	46,75	-0,67	0,45
372	46,75	-0,67	0,45
373	47,5	0,08	0,01
374	47,5	0,08	0,01
375	47,5	0,08	0,01
376	47,5	0,08	0,01
377	44	-3,42	11,70
378	44	-3,42	11,70
379	44	-3,42	11,70
380	44	-3,42	11,70
381	43	-4,42	19,54
382	43	-4,42	19,54
383	43	-4,42	19,54
384	43	-4,42	19,54
385	46	-1,42	2,02

386	46	-1,42	2,02
387	46	-1,42	2,02
388	46	-1,42	2,02
389	44,75	-2,67	7,13
390	44,75	-2,67	7,13
391	44,75	-2,67	7,13
392	44,75	-2,67	7,13
393	48,5	1,08	1,16
394	48,5	1,08	1,16
395	48,5	1,08	1,16
396	48,5	1,08	1,16
397	45	-2,42	5,86
398	45	-2,42	5,86
399	45	-2,42	5,86
400	45	-2,42	5,86
401	46,5	-0,92	0,85
402	46,5	-0,92	0,85
403	46,5	-0,92	0,85
404	46,5	-0,92	0,85
405	44,75	-2,67	7,13
406	44,75	-2,67	7,13
407	44,75	-2,67	7,13
408	44,75	-2,67	7,13
409	49	1,58	2,49
410	49	1,58	2,49
411	49	1,58	2,49
412	49	1,58	2,49
413	49	1,58	2,49
414	49	1,58	2,49
415	49	1,58	2,49

416	49	1,58	2,49
417	50,5	3,08	9,48
418	50,5	3,08	9,48
419	50,5	3,08	9,48
420	50,5	3,08	9,48
421	48,5	1,08	1,16
422	48,5	1,08	1,16
423	48,5	1,08	1,16
424	48,5	1,08	1,16
425	47,5	0,08	0,01
426	47,5	0,08	0,01
427	47,5	0,08	0,01
428	47,5	0,08	0,01
429	48,25	0,83	0,69
430	48,25	0,83	0,69
431	48,25	0,83	0,69
432	48,25	0,83	0,69
433	48,25	0,83	0,69
434	48,25	0,83	0,69
435	48,25	0,83	0,69
436	48,25	0,83	0,69
437	46,5	-0,92	0,85
438	46,5	-0,92	0,85
439	46,5	-0,92	0,85
440	46,5	-0,92	0,85
441	47,5	0,08	0,01
442	47,5	0,08	0,01
443	47,5	0,08	0,01
444	47,5	0,08	0,01
445	47	-0,42	0,18

446	47	-0,42	0,18
447	47	-0,42	0,18
448	47	-0,42	0,18
449	48,25	0,83	0,69
450	48,25	0,83	0,69
451	48,25	0,83	0,69
452	48,25	0,83	0,69
453	46,75	-0,67	0,45
454	46,75	-0,67	0,45
455	46,75	-0,67	0,45
456	46,75	-0,67	0,45
457	48	0,58	0,34
458	48	0,58	0,34
459	48	0,58	0,34
460	48	0,58	0,34
461	48	0,58	0,34
462	48	0,58	0,34
463	48	0,58	0,34
464	48	0,58	0,34
465	41,25	-6,17	38,08
466	41,25	-6,17	38,08
467	41,25	-6,17	38,08
468	41,25	-6,17	38,08
469	45,5	-1,92	3,69
470	45,5	-1,92	3,69
471	45,5	-1,92	3,69
472	45,5	-1,92	3,69
473	46,75	-0,67	0,45
474	46,75	-0,67	0,45
475	46,75	-0,67	0,45

476	46,75	-0,67	0,45
477	48,5	1,08	1,16
478	48,5	1,08	1,16
479	48,5	1,08	1,16
480	48,5	1,08	1,16
Jumlah	**22762,00**		**1658,99**
Rataan	**47,42**		
SD	**1,86**		
KK	**3,92**		

Mean of Standard Deviation

$$SD = \sqrt{\frac{\Sigma(x-\bar{x})2}{n-1}} = \sqrt{\frac{1658.99}{480-1}} = 1,86$$

With =

x = data for each treatment column

$\bar{x}$ = mean

n = total data

$$\text{Coefficient of Variation (CV)} = \frac{(SD)}{mean} x\ 100\%$$

$$= \frac{1.86}{47,42} x\ 100\%$$

$$= 3,92\%$$

Conclusion: The broiler used in this study can be said to be uniform because it has a coefficient of variation less than 10%.

Appendix 2. Analysis the Effect of Treatment on pH of the Small Intestine Digesta

Treatment	Replication						Total	Mean
(Code)	1	2	3	4	5	6		
P0	6,9	6,3	7,4	6,6	7,3	6,2	40,7	6,78 ± 0,5
P1	6,5	6,9	6,9	6,5	6,9	7,5	41,2	6,87 ± 0,37
P2	6,8	6,4	6,7	7,4	7,6	6,5	41,4	6,90 ± 0,49
P3	6,6	6,6	7,1	7,2	7,2	6,8	41,5	6,92 ± 0,29
P4	6,8	6,5	7	6,9	6,1	6,8	40,1	6,68 ± 0,33
Total							204,9	

a. Correction Factor (CF)

CF $= (\sum i \sum j Yij)^2 / (t \times r)$

$= (204,9)^2 / (5 \times 6)$

$= 4198,01 \times 30$

$= 1399,467$

b. Sum of Squares Total (SSY)

SS Total $= \sum i \sum j (Yij)^2$ - CF

$= (6,9^2+6,3^2 +…+ 6,1^2 + 6,8^2)-$ 1399,467

$= 1403,79 - 1399,467$

$= 4,323$

c. Sum of Squares Treatment (SST)

SS Treatment= $\sum i (\sum j Yij)^2 / r$ – CF

$=(40,7^2+41,2^2+…+40,1^2)/6-1399,467$

$= (8398,15) / 6 - 1399,467$

= 1399,692 - 1399,467
= 0,225

d. Sum of Squares Error (SSE)

SS Error = SS Total - SS Treatment
= 4,323 - 0,225
= 4,098

e. Degrees of Freedom Treatment

DF Treatment = t-1
= 5-1
= 4

f. Degrees of Freedom Error

DF Error = t(r-1)
=5(6-1)
= 25

g. Mean Squares Treatment (MS Treatment)

MS Treatment = SS Treatment / df Treatment
= 0,225/ 4
= 0,06

h. Mean Squares Error (MS Error)

MS Error = SSError / df Error
= 4,10 / 25
= 0,16

i. F ratio = MS Treatment / MS Error
= 0,06 / 0,16
= 0,34

ANOVA TABLE

Source of Variation	df	SS	MS	F	F 5%	F 1%
Treatment	4	0,22	0,06	0,34^{ns}	2,76	4,18
Error	25	4,10	0,16			
Total	29	4,32				

Conclusion:

F ratio < Ftable (0,05), showed that the treatment has no different effect (P>0,05) on the pH of the small intestine digesta of Broiler

Appendix 3. Analysis the Effect of Treatment on Viscosity of the Small Intestine Digesta

Treatment (Code)	Replication						Total	Mean
	1	2	3	4	5	6		
P0	4	3	2	4	3	4	20	3.33 ± 0,82
P1	3	3	4	2	4	3	19	3.17 ± 0,75
P2	3	4	3	3	3	3	19	3.17 ± 0,41
P3	3	3,5	2	4	3	3	18,5	3.08 ± 0,66
P4	4	3	4	3	4	3	21	3.50 ± 0,55
Total							97,5	

a. Correction Factor (CF)

$$CF = (\sum i \sum j Yij)^2 / (t \times r)$$
$$= (97,5)^2 / (5 \times 6)$$
$$= 9506,25 \times 30$$
$$= 316,88$$

b. Sum of Squares Total (SSY)

$$SS\ Total = \sum i \sum j (Yij)^2 - CF$$
$$= (4^2 + 3^2 + \ldots + 3^2 + 3^2) - 316,88$$
$$= 328,25 - 316,88$$
$$= 11,38$$

c. Sum of Squares Treatment (SST)

$$SS\ Treatment = \sum i (\sum j Yij)^2 / r - CF$$
$$= (20^2 + 19^2 + \ldots + 21^2) / 6 - 316,88$$
$$= (1905,25) / 6 - 316,88$$

= 317,54 - 316,88
= 0,66

d. Sum of Squares Error (SSE)
SS Error = SS Total - SS Treatment
= 11,38 – 0,66
= 10,708

e. Degrees of Freedom Treatment
DF Treatment = t-1
= 5-1
= 4

f. Degrees of Freedom Error
DF Error = t(r-1)
=5(6-1)
= 25

g. Mean Squares Treatment (MS Treatment)
MS Treatment = SS Treatment / df Treatment
= 0,66/ 4
= 0,167

h. Mean Squares Error (MS Error)
MS Error = SSError / df Error
= 10,708/ 25
= 0,428

i. F ratio = MS Treatment / MS Error
= 0,167 / 0,428
= 0,389

ANOVA TABLE

Source of Variation	df	SS	MS	F	F 5%	F 1%
Treatment	4	0.667	0.167	0.389**	2.76	4.18
Error	25	10.708	0.428			
Total	29	11.375				

Conclusion :

F ratio < Ftable (0,05), showed that treatment has no significant different effect (P>0,05) on the viscosity of small intestine digesta of broiler.

Appendix 4. Analysis the Effect of Treatment on Amylase Enzyme Activity of the Small Intestine

Treat-ment (Code)	Replication						Total	Mean
	1	2	3	4	5	6		
P0	571,8	528,14	571,8	528,14	549,97	549,97	3299,82	549,97 ± 25,21
P1	512,46	491,92	512,46	491,92	502,19	502,19	3013,14	502,19 ± 11,86
P2	712,08	629,57	712,08	629,57	670,825	670,825	4024,95	670,825 ± 47,64
P3	660,5	502,84	660,5	502,84	581,67	581,67	3490,02	581,67 ± 91,03
P4	591,18	504,44	591,18	504,44	547,81	547,81	3286,86	547,81 ± 50,08
Total							17114,79	

a. Correction Factor (CF)

$$CF = \left(\sum_i \sum_j Yij\right)^2 / (t \times r)$$

$$= (17114,79^2) / (5x6)$$

$$= 292916036,7 / 30$$

$$= 9763867,9$$

b. Sum of Squares Total (SSY)

$$SS\ Total = \sum_i \sum_j (Yij)^2 - CF$$

$$= (571,8^2+549,97^2+\ldots+ 547,81^2+ 504,44^2) - 9763867,9$$

$$= 9900139,07 - 9763867,9$$

$$= 136271,18$$

c. Sum of Squares Treatment (SST)

SS Treatment = $\sum i \left(\sum j Yij \right)^2 / r - CF$

= (3299,82^2+ 3013,14^2 + … + 3286,86^2) / 6 - 9763867,9

= 59151735,45/ 6 - 9763867,9

= 94754,684

d. Sum of Squares Error (SSE)

SS Error = SS Total - SS Treatment

= 136271,17 - 94754,684

= 41516,491

e. Degrees of Freedom Treatment

DF Treatment = t-1

= 5-1

= 4

f. Degrees of Freedom Error

DF Error = t(r-1)

=5(6-1)

= 25

g. Mean Squares Treatment (MS Treatment)

MS Treatment = SS Treatment / df Treatment

= 94754,684 / 4

= 23688,671

h. Mean Squares Error (MS Error)

MS Error = SSError / df Error

= 41516,491 / 25

= 1660,660

i. F ratio = MS Treatment / MS Error
= 23688,671 / 1660,660
= 14,265

ANOVA Table

Source of Variation	df	SS	MS	F	F 5%	F 1%
Treatment	4	94754,684	23688,67	14,26**	2,759	4,177
Error	25	41516,491	1660,660			
Total	29	136271,175				

Conclusion :

F ratio > Ftable (0,01), showed that the treatment has highly significant different effect (P<0,01) on amylase enzyme activity of small intestine broiler. There is a highly significant different effect, then follow-up with DMRT to determine the difference effects between treatments.

Further test DMRT

DMRT 1% = Duncan Critical Value x SE

$= \text{Duncan Critical Value} \times \sqrt{\frac{MS\ Error}{Replication}}$

DMRT 1% = (3,94 ; 4,11 ; 4,23 ; 4,31) x $\sqrt{\frac{1660,660}{6}}$

= (3,94 ; 4,11 ; 4,23 ; 4,31) x 16,64

p	Duncan Critical Value (1%)	DMRT
2	3,94	65,58
3	4,11	68,41
4	4,23	70,29
5	4,31	71,65

DMRT Result of amylase enzyme activity of small intestine broiler

Treatment	Mean	Notation
P2	670.825	A
P3	581.67	B
P0	549.97	BC
P4	547.81	C
P1	502.19	C

Different superscript letters (A-D) in the same row showed a significantly different effect (P<0,01) on amylase activity enzyme.

Appendix 5. Analysis the Effect of Treatment on Protease Enzyme Activity of the Small Intestine

Treatment (Code)	Replication 1	2	3	4	5	6	Total	Mean
P0	5,19	5,23	5,21	5,21	5,19	5,23	31,26	5,210 ± 0,016
P1	5,6	4,39	5,00	5,00	5,6	4,39	29,97	4,995 ± 0,493
P2	4,39	3,5	3,95	3,95	4,39	3,5	23,67	3,945 ± 0,363
P3	5,3	7,93	6,62	6,62	5,3	7,93	39,69	6,615 ± 0,073
P4	7,8	7,98	7,89	7,89	7,8	7,98	47,34	7,890 ± 0,306
Total							171,93	

a. Correction Factor (CF)

$$CF = \left(\sum_i \sum_j Yij\right)^2 / (t \times r)$$

$$= (171,93^2) / (5x6)$$

$$= 29559,925 / 30$$

$$= 985,331$$

b. Sum of Squares Total (SSY)

$$SS\ Total = \sum_i \sum_j (Yij)^2 - CF$$

$$= (5,19^2 + 5,23^2 + \ldots + 7,8^2 + 7,98^2) - 985,331$$

$$= 1051,211 - 985,331$$

$$= 65,881$$

c. Sum of Squares Treatment (SST)

$$SS\ Treatment = \sum_i \left(\sum_j Yij\right)^2 / r - CF$$

= (31,26^2 + 29,97^2 + … + 47,34^2)
/ 6 - 985,331
= 6252,029 / 6 - 985,331
= 56,674

d. Sum of Squares Error (SSE)

SS Error = SS Total - SS Treatment
= 65,881 - 56,674
= 9,207

e. Degrees of Freedom Treatment

DF Treatment = t-1
= 5-1
= 4

f. Degrees of Freedom Error

DF Error = t(r-1)
=5(6-1)
= 25

g. Mean Squares Treatment (MS Treatment)

MS Treatment = SS Treatment / df Treatment
= 56,674 / 4
= 14,169

h. Mean Squares Error (MS Error)

MS Error = SSError / df Error
= 9,207 / 25
= 0,368

i. F ratio = MS Treatment / MS Error

= 14,169/ 0,368

= 38,472

ANOVA Table

Source of Variation	df	SS	MS	F	F 5%	F 1%
Treatment	4	56,674	14,169	38,472**	2,759	4,177
Error	25	9,207	0,368			
Total	29	65,881				

Conclusion :

F ratio > Ftable (0,01), showed that the treatment has highly significant different effect (P<0,01) on protease enzyme activity of small intestine broiler. There is a highly significant different effect, then follow-up with DMRT to determine the difference effects between treatments.

Further test DMRT

DMRT 1% = Jarak Duncan x $\sqrt{\frac{MS\ Error}{Replication}}$

DMRT 1% = (3,94 ; 4,11 ; 4,23 ; 4,31) x $\sqrt{\frac{0,384}{6}}$

= (3,94 ; 4,11 ; 4,23 ; 4,31) x 0,25

p	Duncan Critical Value (1%)	DMRT (1%)
2	3,94	0,98
3	4,11	1,02
4	4,23	1,05
5	4,31	1,07

DMRT Result of protease enzyme activity of small intestine broiler

Treatment	Mean	Notation
P4	7,89	A
P3	6,615	B
P0	5,21	C
P1	4,995	CD
P2	3,945	D

Different superscript letters (A-D) in the same row showed a significantly different effect (P<0,01) on protease activity enzyme

Appendix 6. Analysis the Effect of Treatment on the Excreta Ammonia Level

Treatment (Code)	Replication				Total	Mean
	1	2	3	4		
P0	0,05	0,01	0,11	0,3	0,47	0,118 ± 0,128
P1	0,17	0,19	0,18	0,39	0,93	0,233 ± 0,105
P2	0,12	0,6	0,13	0,1	0,95	0,238 ± 0,242
P3	0,29	0,55	0,3	0,2	1,34	0,335 ± 0,150
P4	0,6	0,31	0,3	0,405	1,615	0,404 ± 0,139
Total	1,23	1,66	1,02	1,395	5,305	0,265

a. Correction Factor (CF)

$$CF = \left(\sum_i \sum_j Yij\right)^2 / (t \times r)$$

$$= (5{,}305^2) / (5 \times 4)$$

$$= 28{,}143 / 20$$

$$= 1{,}407$$

b. Sum of Squares Total (SSY)

$$SS\ Total = \sum_i \sum_j (Yij)^2 - CF$$

$$= (0{,}05^2 + 0{,}01^2 + \ldots + 0{,}3^2 + 0{,}405^2) - 1{,}407$$

$$= 1{,}982 - 1{,}407$$

$$= 0{,}575$$

c. Sum of Squares Treatment (SST)

$$SS\ Treatment = \sum_i \left(\sum_j Yij\right)^2 / r - CF$$

$$= (0{,}47^2 + 0{,}93^2 + \ldots + 1{,}615^2) / 4 - 1{,}407$$

= 6,392 / 4 - 1,407
= 0,191

d. Sum of Squares Error (SSE)
SS Error = SS Total - SS Treatment
= 0,575 – 0,191
= 0,384

e. Degrees of Freedom Treatment
DF Treatment = t-1
= 5-1
= 4

f. Degrees of Freedom Error
DF Error = t(r-1)
=5(4-1)
= 15

g. Mean Squares Treatment (MS Treatment)
MS Treatment = SS Treatment / df Treatment
= 0,191/ 4
= 0,048

h. Mean Squares Error (MS Error)
MS Error = SSError / df Error
= 0,384 / 15
= 0,026

i. F ratio = MS Treatment / MS Error
= 0,048 / 0,026
= 1,863

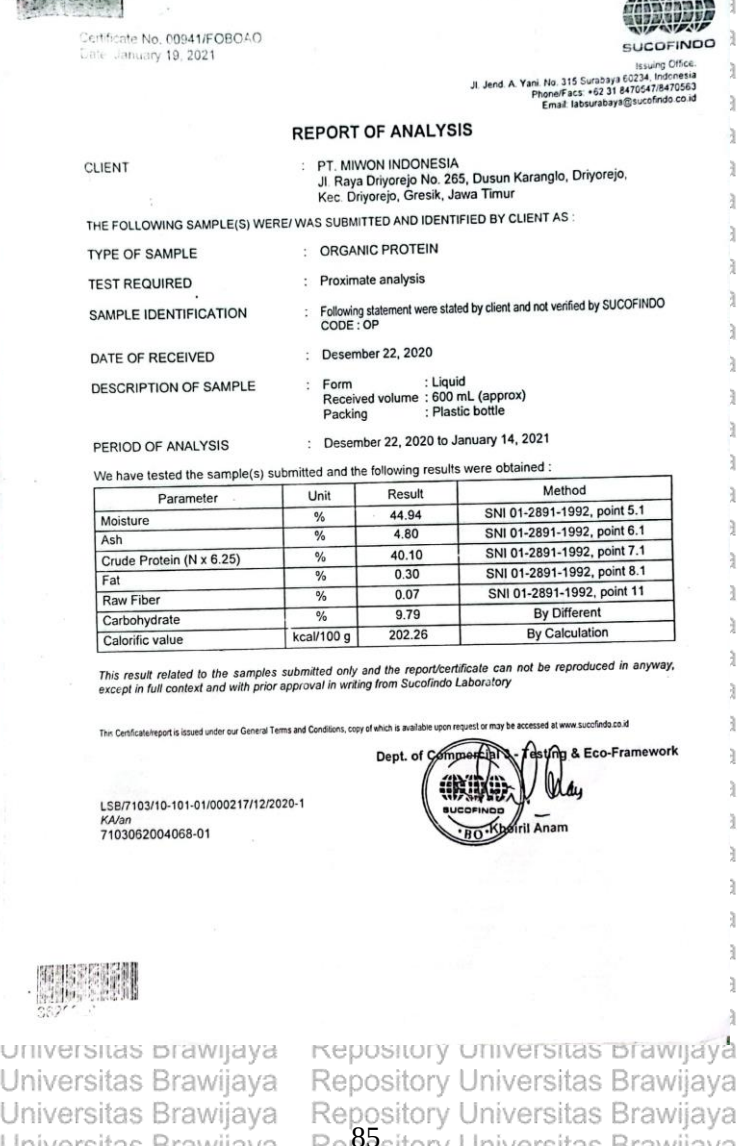
ANOVA Table

Source of Variation	df	SS	MS	F	F 5%	F 1%
Treatment	4	0,191	0,048	1,863	3,056	4,893
Error	15	0,384	0,026			
Total	19	0,575				

Conclusion:

F ratio < Ftable (0,05), showed that the treatment has no significant different effect (P>0,05) on ammonia levels of broiler excreta.

Appendix 7. Organik protein Analysis Result



Certificate No. 00941/FOBOAO
Date: January 19, 2021

SUCOFINDO
Issuing Office:
Jl. Jend. A. Yani No. 315 Surabaya 60234, Indonesia
Phone/Facs: +62 31 8470547/8470563
Email: labsurabaya@sucofindo.co.id

REPORT OF ANALYSIS

CLIENT : PT. MIWON INDONESIA
Jl. Raya Driyorejo No. 265, Dusun Karanglo, Driyorejo,
Kec. Driyorejo, Gresik, Jawa Timur

THE FOLLOWING SAMPLE(S) WERE/ WAS SUBMITTED AND IDENTIFIED BY CLIENT AS :

TYPE OF SAMPLE : ORGANIC PROTEIN

TEST REQUIRED : Proximate analysis

SAMPLE IDENTIFICATION : Following statement were stated by client and not verified by SUCOFINDO
CODE : OP

DATE OF RECEIVED : Desember 22, 2020

DESCRIPTION OF SAMPLE : Form : Liquid
Received volume : 600 mL (approx)
Packing : Plastic bottle

PERIOD OF ANALYSIS : Desember 22, 2020 to January 14, 2021

We have tested the sample(s) submitted and the following results were obtained :

Parameter	Unit	Result	Method
Moisture	%	44.94	SNI 01-2891-1992, point 5.1
Ash	%	4.80	SNI 01-2891-1992, point 6.1
Crude Protein (N x 6.25)	%	40.10	SNI 01-2891-1992, point 7.1
Fat	%	0.30	SNI 01-2891-1992, point 8.1
Raw Fiber	%	0.07	SNI 01-2891-1992, point 11
Carbohydrate	%	9.79	By Different
Calorific value	kcal/100 g	202.26	By Calculation

This result related to the samples submitted only and the report/certificate can not be reproduced in anyway, except in full context and with prior approval in writing from Sucofindo Laboratory

This Certificate/report is issued under our General Terms and Conditions, copy of which is available upon request or may be accessed at www.sucofindo.co.id

Dept. of Commercial Testing & Eco-Framework

LSB/7103/10-101-01/000217/12/2020-1
KA/an
7103062004068-01

SUCOFINDO
BO Khairil Anam

Appendix 8. Feed Analysis Result

PEMERINTAH KABUPATEN BLITAR
DINAS PETERNAKAN DAN PERIKANAN
JalanCokroaminoto No. 22 Telp (0342) 801136 Fax. (0342) 801136 Blitar 66112
Email : disnakkan@blitarkab.go.id

KAN Komite Akreditasi Nasional Laboratorium Penguji LP-1243-IDN

LAPORAN HASIL PENGUJIAN
NO. LHP F.62.PKN/VI/2021

Asal Sampel : Osfar
Alamat : Malang,Jawa Timur
No. Telp. / HP : 085735287300
Nomor Surat : 524.6/62.PKN. VI/409.115.3/2021
Jenis Sampel : Pakan
Keterangan kondisi sampeL : 6 (enam) sampel dalam kondisi baik

Tanggal diterima : 15 Juni 2021 (10.39 WIB)
Analisa/ uji : Proksimat (Air, Abu, Protein Kasar, Lemak Kasar dan Sera Kasar) dan Gross Energy
Nomor Permintaan Uji : F.62 (01-06)
Tanggal Mulai Pengujian : 16 Juni 2021
Tanggal Selesai Pengujian : 08 Juli 2021(14.30 WIB)
Tanggal LHP : 08 Juli 2021(14.40-15.20 WIB)

No	No. Uji	JENIS SAMPEL	BAHAN KERING (%)	AIR (%)		ABU (%)		PROTEIN KASAR (%)		LEMAK KASAR (%)		SERAT KASAR (%)		KALSIUM (%)		FOSFOR (%)		GROSS ENERGY (cal/g)
			Sampel	Sampel	SNI (maks)	Sampel	SNI (maks)	Sampel	SNI (min)	Sampel	SNI (maks)	Sampel	SNI (maks)	Sampel	SNI (min)	Sampel	SNI (min)	Sampel
1	F.62.01	BRI C P0	89,89	10,11	14	4,98	8	22,44	20	6,37	Min. 5	**8,07**	5					4164
2	F.62.02	BRI C P1	89,33	10,67	14	5,15	8	23,71	20	6,02	Min. 5	4,83	5					4128
3	F.62.03	BRI C P2	88,43	11,57	14	5,35	8	23,04	20	5,41	Min. 5	**5,13**	5					4040
4	F.62.04	BRI C P3	86,10	13,90	14	5,35	8	23,43	20	5,47	Min. 5	4,59	5					4023
5	F.62.05	BRI C P4	89,20	**14,80**	14	5,53	8	23,40	20	5,89	Min. 5	4,60	5					3968
6	F.62.06	PBI C P0	92,62	7,38	14	5,31	8	20,63	20	5,23	Min. 5	4,83	5					4082
METODE				AOAC 2016, Bab 4 Butir 4.1.06. Metode 930.15		AOAC 2016, Bab 4 Butir 4 1.10. Metode 942.05		AOAC 2016, Bab 4 Butir 4.2.11. Metode 2001.11		SNI.01.2891-1992. Butir 8.1		SNI.01-2891-1992. Butir 11		AOAC 2016, Bab 4 Butir 4.8.03 metode 927.02		AOAC 2016, Bab 4 Butir 4.8.14 Metode 965.17		Kalorimetri

Keterangan :
- Angka yang dicetak tebal dan bergaris bawah tidak sesuai dengan Standar Nasional Indonesia (SNI)
- Hasil uji sampel NO1-6 disesuaikan dengan SNI 8173.2:2015 (Pakan Ayam Ras Pedaging Masa Awal (*Broiler Stater*))
- Hasil uji sampel berdasarkan pemeriksaan sampel yang diterima/ *as feed*
  *) Ruanglingkup parameter pengujiantidakmasukakreditasi

PENYELIA

Dhani Ambarwati, S.Pt
NIP. 19800116 200604 2 021

LABORATORIUM PAKAN LABORATORIUM PAKAN LABORATORIUM PAKAN

Putih : Pelanggan, Merah : Arsip

Appendix 9. Research Documentation

Preparing Bamboo Cage

Cage disinfection

Treatment Feed

DOC

Brown Sugar Drink

ND vaccine

Gumboro Vaccine

Scale

Commercial Feed

Feeder and Drinker

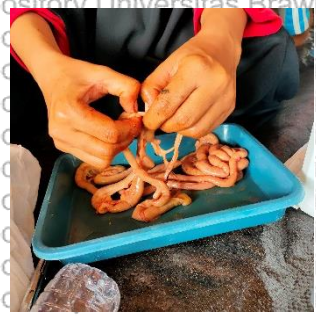
Laboratory Equipment

DOC weighing

1st Week Weighing

Broiler in 2nd week

5th week weighing

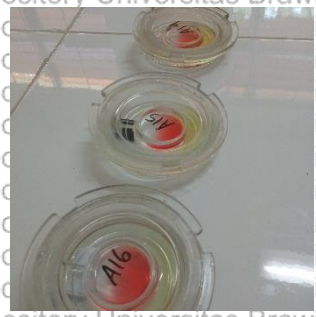


Digesta Sampling

Excreta Sampling

Centrifugation

Viscosity test



Conway Tes

Ammonia Supernatant

Organik protein